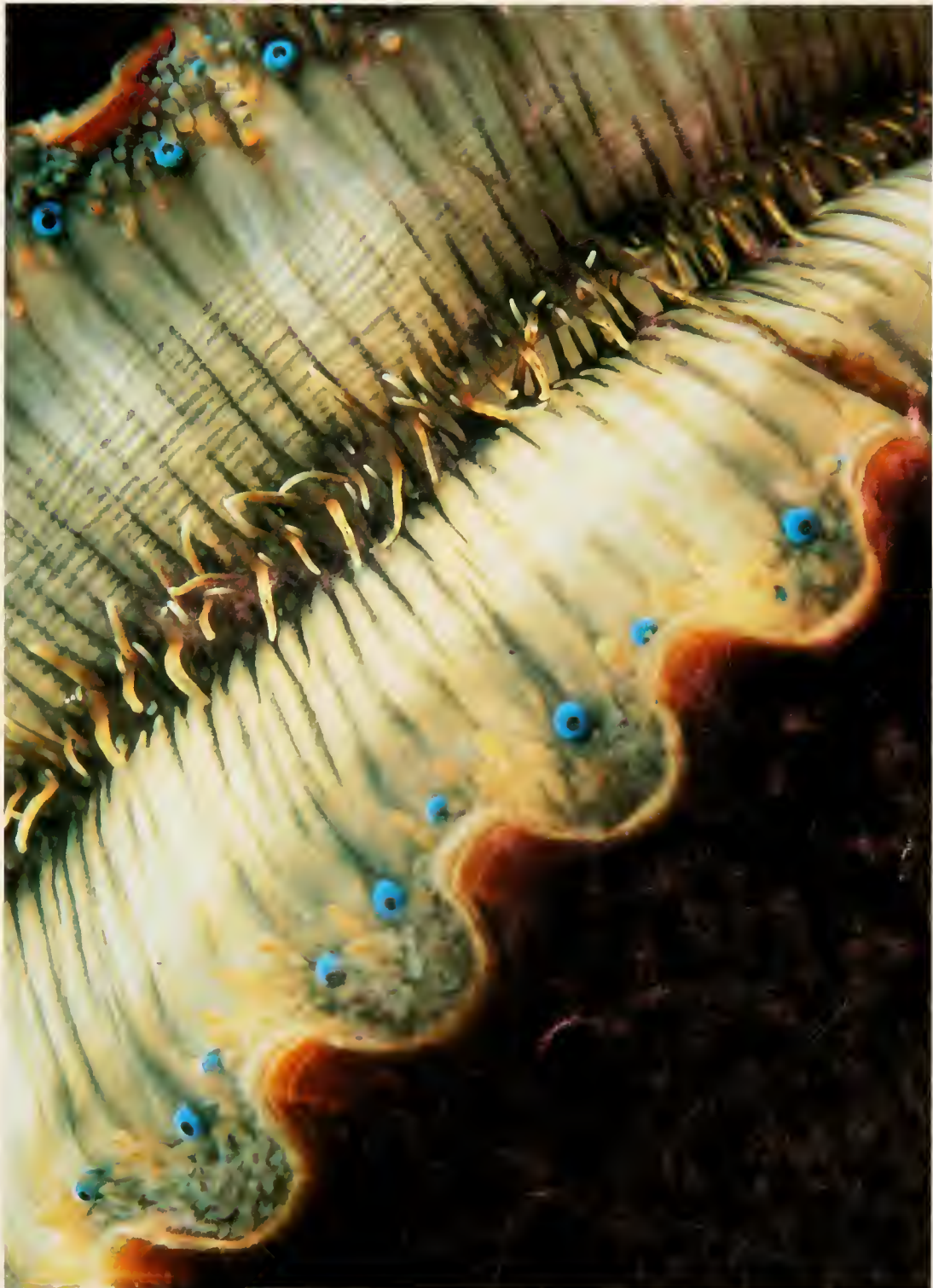


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PREDATION OF JUVENILE SEA SCALLOPS (*PLACOPECTEN MAGELLANICUS*) BY CRABS (*CANCER IRRORATUS* AND *HYAS* SP.) AND STARFISH (*ASTERIAS VULGARIS*, *LEPTASTERIAS POLARIS*, AND *CROSSASTER PAPPOSUS*)

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APR 13 1999

ABSTRACT A scallop fishermen's association has seeded sea scallops, *Placopecten magellanicus*, off Îles-de-la-Madeleine (Québec, Canada) since 1993. When the scallops reach the bottom, they fall prey to starfish and crabs. Two size classes are available during the Îles-de-la-Madeleine seeding period: small scallops from collectors (15 to 25 mm shell height) and large scallops grown-out in pearl nets (35 to 45 mm). To evaluate predation on both classes shortly after seeding, experiments were performed under controlled laboratory conditions. Testing involved three species of starfish, *Asterias vulgaris* (120 to 200 mm diameter), *Leptasterias polaris* (120 to 200 mm diameter), and *Crossaster papposus* (110 to 130 mm diameter), and two species of crab, *Cancer irroratus* (85 to 120 mm carapace width) and *Hyas* sp. (90 to 110 mm carapace length). Scallops from both size classes were presented separately (nonchoice treatment) and together (choice treatment) to each predator species. Starfish and *Hyas* sp. consumed less than one scallop per predator per day as compared to *C. irroratus*, which consumed as many as 12 scallops per predator per day. Starfish and crabs did not show a clear prey size preference in both treatments. However, large scallops tend to be consumed faster by both crab species in choice treatments. These results indicate that bottoms with high densities of starfish or crab should be avoided in seeding. In addition, bottom seeding should be done if possible with scallops greater than the size classes used in this study (over 50 mm), according on these results and those of other studies.

KEY WORDS: *Placopecten magellanicus*, scallop, enhancement, predation

INTRODUCTION

Sea scallops, *Placopecten magellanicus* (Gmelin), have been seeded commercially off Îles-de-la-Madeleine (Québec, Canada) in the Gulf of St-Lawrence since 1993. The profitability of these operations hinges on scallop survival rates 4 years after seeding, until scallops reached the commercial shell height of 90 mm. Predation is an important factor affecting the survival of juvenile scallops between seeding and harvest. In Nova Scotia, Hatcher et al. (1996) showed that 50% of their seeded scallops were killed by crabs and starfish within 2 weeks. Experimental summer and winter seedings performed by Barbeau et al. (1996) indicated survival rates of 1 and 10%, respectively, after only 8 weeks. Crab (*Cancer irroratus* Say) predation was identified as the prime factor of scallop mortalities. Cliche et al. (1994) determined during an experimental seeding performed in Îles-de-la-Madeleine that 11.5% of seeded scallops were killed by crabs within 44 days. Haugum et al. (1997) lost all of their seeded scallops to crab (*Cancer pagurus*) predation within 3 weeks during an experimental bottom seeding in Norway.

Many factors may affect the impact of predation after seeding. Size of seeded scallops is generally considered important (Elner and Jamieson 1979, Morgan et al. 1980, Lake and Jones 1987, Minchin 1991, Barbeau and Scheibling 1994a, Barbeau et al. 1994, Arsenault and Himmelman 1996). In Îles-de-la-Madeleine, two size classes are seeded in late fall. Scallops kept in collector bags attain 15 to 25 mm shell height after 1 year, and those transferred to pearl nets, 35 to 45 mm. Production costs (labor and equipment costs) of scallops grown-out in pearl nets are five times higher than scallops grown directly in collector bags. Thus, it is essential to estimate the survival rate of both classes when choosing an optimal growing strategy.

Previous surveys around Îles-de-la-Madeleine showed that starfish are abundant (~0.5 starfish/m²) in natural scallop grounds, with *Asterias vulgaris* (Verrill) and *Leptasterias polaris* (Müller et

Troschel) predominating and *Crossaster papposus* (L.) regularly observed. Low densities of two crab species (~0.05 crabs/m²), *C. irroratus* and *Hyas* sp., are also reported. Predation of juvenile scallops by *A. vulgaris* and *C. irroratus* has been documented by Elner and Jamieson (1979), Jamieson et al. (1982), Lake et al. (1987), Barbeau and Scheibling (1994a), Barbeau and Scheibling (1994b), Barbeau et al. (1994), and Arsenault and Himmelman (1996). However, few studies have examined predation involving sea scallops (*P. magellanicus*) of 15 to 50 mm shell height (Elner and Jamieson 1979). Barbeau and Scheibling (1994a) and Barbeau and Scheibling (1994b) and Barbeau et al. (1994) used scallops of 5 to 28 mm shell height, and Jamieson et al. (1982) used scallops of 40 to 55 mm and 80 to 110 mm. In addition, very little documentation exists on the predatory capacity of *L. polaris*, *C. papposus* and *Hyas* sp. on scallops (Arsenault and Himmelman 1996).

Scallop vulnerability is high the first few days after seeding (Cliche et al. 1994, Barbeau et al. 1996). Stress induced during handling, exposure to air, and transportation to the seeding site may affect scallop vitality (Fleury et al. 1996). After seeded scallops reach the bottom, the time required to turn up their superior valve and find refuge may have an impact on their survival. Arsenault and Himmelman (1996) concluded that refuge use decreased the risk of predation for smaller scallops. Thus, studies conducted to increase the seeded scallops survival must target this period in particular.

The objective of this study was to evaluate the impact or predation by starfish, *A. vulgaris*, *L. polaris* and *C. papposus* and crabs, *C. irroratus* and *Hyas* sp., on small scallops from collector bags and larger ones from pearl nets under controlled conditions. This information is important when planning a seeding bottom strategy for a profitable commercial operation.

MATERIALS AND METHODS

Experiments were conducted in 2,000-L (1.1 m × 2.3 m × 0.7 m) and 1,500-L (1.1 m × 1.6 m × 0.7 m) fiber glass tanks with

circulating sea water. Temperature was maintained at $11.3 \pm 3.0^\circ\text{C}$ (mean \pm SD), oxygen at 8.4 ± 0.7 mg/L, and salinity at 30.3 ± 0.9 ppt. A 12:12 light:dark regime was simulated by fluorescent lights over each tank (~ 250 lux). Red bulbs were used to allow video recording in the dark, as used in Barbeau and Scheibling's (1994b) experiments.

The cultivated juvenile scallops were taken from commercial operations of the scallop fishermen's association of Îles-de-la-Madeleine. Specimens measuring 15 to 25 mm shell height came from spat collectors, and those measuring 35 to 45 mm from intermediate culture in pearl nets. The shell height was measured from the middle of the dorsal hinge to the farthest point of the ventral shell edge. A plastic tag (glue-on flexible polyethylene shellfish tag, Hallprint Ltd) of 4×8 mm was glued with cyanoacrylate glue on the upper valve. *C. irroratus* and *Hyas* sp. were caught with rock crab or lobster traps. *C. irroratus* averaged 109.8 ± 6.6 mm (mean \pm SD, $n = 45$, range = 95 to 120 mm) carapace width, and *Hyas* sp. were 96.4 ± 7.7 mm ($n = 6$, range = 85 to 110 mm) carapace length. Similar to Barbeau and Scheibling's (1994a) experiments, only male crabs were used to eliminate potential sex-related biases (differences in morphology and predation behavior). Starfish *A. vulgaris* of 153.3 ± 15.9 ($n = 47$, range = 120 to 200 mm) diameter, *L. polaris* of 151.9 ± 21.2 ($n = 45$, range = 120 to 200 mm), and *C. papposus* of 118.5 ± 6.6 mm ($n = 6$, range = 110 to 130 mm diameter) were collected by divers and scallop drags. The size classes of each predator species were selected according to the most abundant predator class evaluated at the actual seeding sites (Roussy et al. 1994).

Scallops, crabs, and starfish were maintained in 650-L seawater tanks for 2 to 10 weeks before testing began. Each species was kept in separate baskets. Scallops were fed on phytoplankton (*Monochrysis lutheri* and *Thalassiosira pseudonana*) at concentra-

tions of 5×10^3 cells/mL. Each predator was fed once a week on two living mussels (*Mytilus edulis*) weighing 10 to 20 g. Before experiments, predators were starved for 72 hours to standardize hunger levels.

Two series of experiments were performed. The first was conducted between August and October 1994 in two 2,000-L tanks, divided using plexiglass separators to obtain four experimental sections of 0.8 m^3 (Table 1). Predatory activity of *C. irroratus*, *A. vulgaris*, and *L. polaris* was evaluated and compared. Three predators of the same species were placed with 16 scallops of either 15 to 25 mm or 35 to 45 mm (nonchoice treatment) or eight scallops of each class together (choice treatment). Predator density was $2.4/\text{m}^2$. Scallop density was similar to the commercial seeding target density, $10/\text{m}^2$. Control treatment involved eight scallops of each class without predator. Each treatment was repeated three times over a maximum of 5 days. In crab treatments, replicates were stopped earlier if all scallops had died.

A video camera (Panasonic, Lunar Lite) fitted over the tanks filmed only one replicate of each experimental treatment because of logistical limitations. Starfish behavior was recorded up to 88 hours and crab behavior, up to 22 hours, because the latter was more active. During frame analysis, the time each predator devoted to searching for prey and the number of encounters between predator and prey were noted. Prey search by starfish was defined as displacement on the tank floor toward scallops with arms tips turned up. Because prey search by crabs was more difficult to determine, all crab movement was considered searching behavior. Any contact between predator and prey was considered an encounter. The capture occurred when starfish arms attached a scallop with their tubefeet or when crabs chelae grabbed a scallop. The number of active scallop escapes after encounters was also counted. An active escape was noted when scallops jumped or

TABLE 1.
Laboratory experiments performed from August to October 1994 and June to November 1995.

Predator Species	Experimental Year	Treatment	Tank or Section Size (m^3)	n Replicate	n prey/replicate		Predator/Replicate	
					Small	Large	n	Size (mm) Mean \pm SD
<i>A. vulgaris</i>	1994	Nonchoice	0.8	3	16	—	3	148.2 \pm 12.6
		Nonchoice	0.8	3	—	16	3	
		Choice	0.8	3	8	8	3	
	1995	Nonchoice	1.8	2	24	—	2	160.9 \pm 17.5
		Nonchoice	1.8	2	—	24	2	
		Choice	1.8	2	12	12	2	
<i>L. polaris</i>	1994	Choice	1.2	4	8	8	2	146.4 \pm 21.6
		Nonchoice	0.8	3	16	—	3	
		Nonchoice	0.8	3	—	16	3	
	1995	Choice	0.8	3	8	8	3	163.5 \pm 15.4
		Nonchoice	1.8	3	24	—	2	
		Nonchoice	1.8	3	—	24	2	
<i>C. papposus</i>	1995	Choice	1.2	3	8	8	2	118.5 \pm 6.6
		Nonchoice	0.8	3	16	—	3	108.8 \pm 6.7
<i>C. irroratus</i>	1994	Nonchoice	0.8	3	—	16	3	
		Choice	0.8	3	8	8	3	
		Nonchoice	0.8	3	—	16	3	
	1995	Nonchoice	1.8	2	24	—	2	112.1 \pm 6.0
		Nonchoice	1.8	2	—	24	2	
		Choice	1.8	2	12	12	2	
<i>Hyas</i> sp.	1995	Choice	1.2	3	8	8	2	96.4 \pm 7.7
		Choice	1.2	3	8	8	2	

swam away from starfish or crabs. Passive escape, noted when a scallop closed its valves without displacement in a predator contact, was impossible to detect, because the video camera was too far from the subject. The number of retractions defined by a retreat of a predator after an encounter was noted.

The second series of experiments was performed between June and November 1995. Some of the trials aimed to repeat the 1994 experiments in two 2,000-L tanks. However, improvements were made to the 1994 experimental design. To increase the surface to 1.8 m², no separators were used. Predator (*C. irroratus*, *A. vulgaris*, and *L. polaris*) density was reduced to 0.8/m² to simulate natural densities more closely. Observations were extended to 13 days to collect more information on starfish predation. Scallops available from commercial operations were larger (15 to 30 mm and 35 to 50 mm) than in the 1994 tests. Experimental treatments consisted of putting two predators of the same species with 24 scallops from one or both size classes (12 of both classes). A control treatment was conducted with 12 scallops of 15 to 30 mm and 12 scallops of 35 to 50 mm without predator. Number of replicates ranged from two to three.

Second, the predatory activities of crab *Hyas* sp. and starfish *C. papposus* were compared, respectively, with those of *C. irroratus* and *A. vulgaris* in two 1,500-L tanks that offered surface of 1.2 m². Predator density was 1.1/m² and scallop density, 10/m². For each replicate, two predators were placed in a tank with eight scallops of 15 to 30 mm and eight scallops of 35 to 50 mm. Control treatment used eight scallops of 15 to 30 mm and eight scallops of 35 to 50 mm without predator. Testing ran a maximum of 13 days but was stopped earlier if all scallops had died. Each treatment was repeated three or four times.

Sequence of experiments and tank allocation for each replicate were random. To simulate seeding conditions, predators were immersed 24 hours before the scallops. During daily observations, dead scallops were removed. The chi-square test was used to compare daily mortality in both size classes. Contingency tables (treatment \times mortality) were prepared, and cells with insufficient data for test validity were grouped. Fisher's exact test was performed if the number of data was still lower than five (Sherrer 1984). Predation rates (number of scallops consumed per predator per 24 hours) were evaluated. All statistical analyses were performed using SAS (1982), version 6.03 software. Data collected during video recording (encounter, capture, escape, and retraction rates) were considered more as an indication, given the absence of replicates.

RESULT

Scallop mortality was related to predation, because no mortality occurred in control treatments during 1994 and 1995 experiments.

Starfish

Video recordings from 1994 showed that starfish spent less than 10% of their time searching for prey in both treatments, remaining immobile on the tank walls the rest of the time. Encounter rates for *A. vulgaris* and prey were between 0.9 to 4.1 per day (Fig. 1). Contacts tended to be higher with large prey in both treatments. *L. polaris* encountered 0.1 to 3.3 prey per day. Contacts between *L. polaris* and small scallops occurred more often. Active scallop escapes were higher for treatments involving *A. vulgaris* (0.8 to 4.0 per predator per day), and larger prey tended to escape more frequently. Predator retraction was noted with *L. polaris* (0.1 to 1.5 per predator per day) and occurred primarily after encoun-

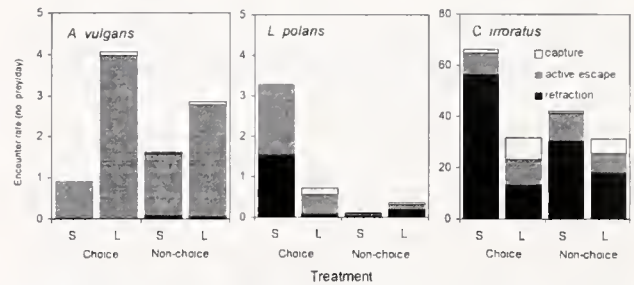


Figure 1. Behavior of small (S) and large (L) scallops in the presence of *Asterias vulgaris*, *Leptasterias polaris*, and *Cancer irroratus* in choice and nonchoice treatments during 1994 experiments.

ters involving smaller prey in choice treatment. All of these factors resulted in low capture rates during recording periods (0 to 0.06 per predator per day).

In nonchoice treatments in 1994, *A. vulgaris* consumed more larger scallops after 4 days 0.22 ± 0.13 (mean \pm SD) per predator per day than smaller ones, 0.03 ± 0.13 ($p = .03$) (Fig. 2). However, when size classes were presented together, mortality rates were similar (0.13 ± 0.12 per predator per day) for both ($p = 0.99$). In contrast, in 1995, only small scallops were consumed in nonchoice treatments after 13 days, with a predation rate of $0.37 \pm$

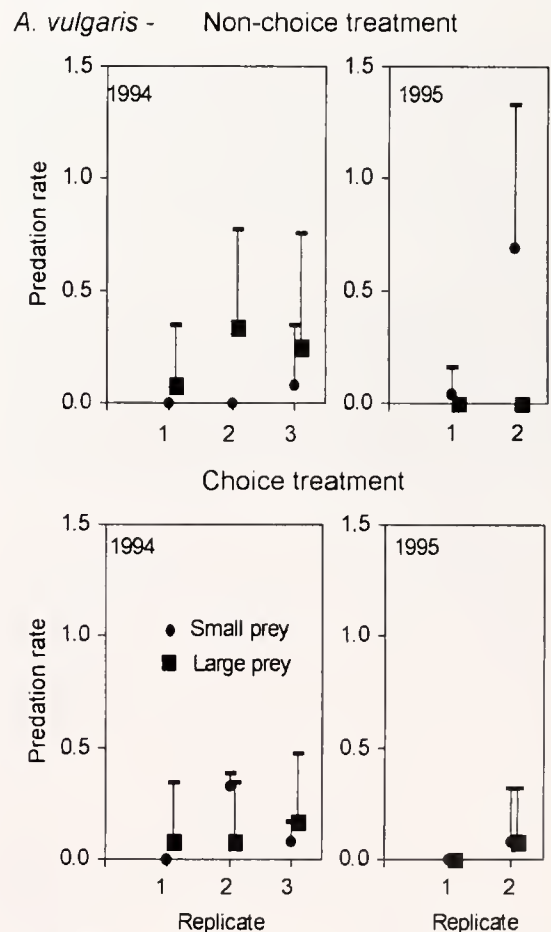


Figure 2. Mean predation rates (n scallops consumed/predator/24 hours) (\pm 95% C.I.) of *Asterias vulgaris* on small and large scallops in choice and nonchoice treatments per replicate during 1994 and 1995 experiments.

0.45. Statistical analysis revealed a difference between mortality rates for both size classes ($p = .001$). In choice treatments, mortality rates were similar for both classes, 0.04 ± 0.05 per predator per day.

Comparison between *C. papposus* and *A. vulgaris* in 1995 choice experiments (Fig. 3), showed that *A. vulgaris* consumed more scallops ($p = .04$) after 13 days. However, both starfish demonstrated similar predation rates on 15 to 30 mm and 35 to 50 mm scallops ($p > .05$). During this experiment, *A. vulgaris* consumed more small scallops, 0.35 ± 0.37 per predator per day, than larger ones, 0.1 ± 0.12 ($p = .002$). *C. papposus* induced similar mortality rates ($p = .06$) on both classes, with predation of 0.05 ± 0.02 .

Experiments performed in 1994 showed that *L. polaris* consumed only larger scallops in nonchoice treatments after 4 days, with a predation rate of 0.42 ± 0.33 (Fig. 4). Predation on both size classes was statistically different ($p = .03$). In choice treatments, predation rates on both classes were similar, 0.10 ± 0.11 ($p > 0.99$). In 1995, nonchoice treatments showed that predation rates for small scallops (0.56 ± 0.34) were higher than those for larger scallops (0.08 ± 0.13) after 13 days ($p = .001$). In choice treatments, smaller scallop mortality was significantly higher (0.24 ± 0.18 per predator per day) than larger scallop mortality (0.08 ± 0.04) ($p = .004$).

Crabs

Video analysis showed that *C. irroratus* spent 70 to 90% of the recorded time searching for prey. Encounter rates between predator and prey were high (31 to 66). Some 7 to 11 active prey escapes occurred per predator per day. Retraction rates were noted 13 to 56 times per predator per day. Encounter and retraction rates tended to be higher with small scallops, but capture rates were higher for larger scallops.

In both experimental years, *C. irroratus* consumed almost all juvenile scallops available in a few days. Consequently, mortality rates were similar for both classes ($p > .05$) at the end of each replicate. Statistical comparison was performed 1 day after seeding to identify a preference for a particular class. In the 1994 experiments, predation rates after 24 hours in nonchoice treatments involving larger scallops were 4.22 ± 0.84 as compared to 2.89 ± 1.07 for smaller ones (Fig. 5). Predation rates in choice treatments

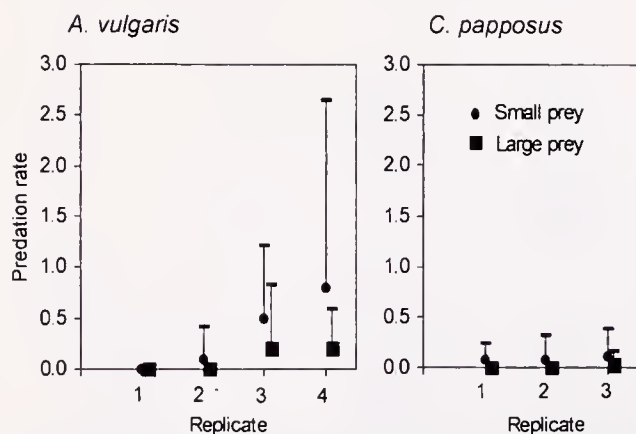


Figure 3. Mean predation rates (n scallops consumed/predator/24 hours) ($\pm 95\%$ C.I.) of *Asterias vulgaris* and *Crossaster papposus* on small and large scallops in choice treatments per replicate during 1995 experiments.

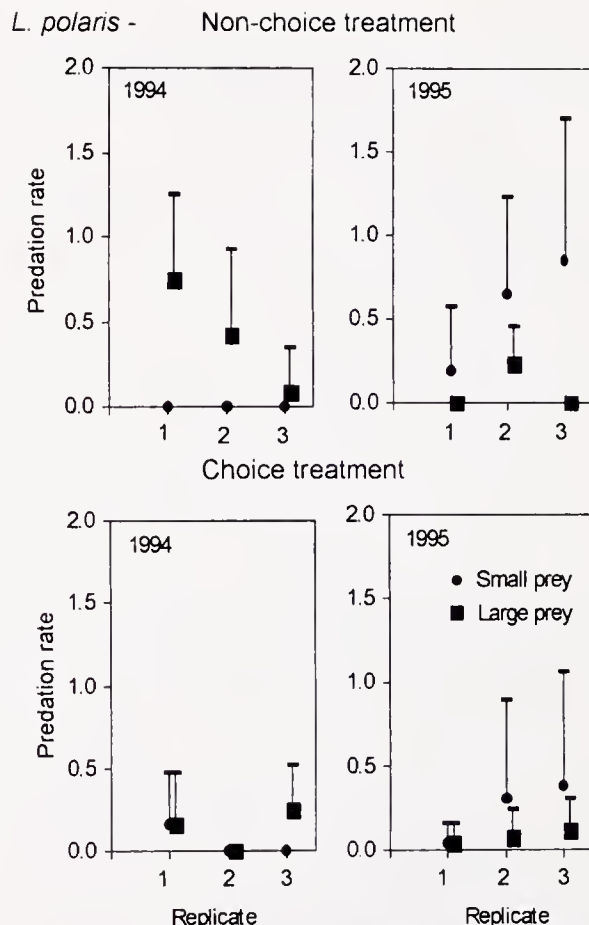


Figure 4. Mean predation rates (n scallops consumed/predator/24 hours) ($\pm 95\%$ C.I.) of *Leptasterias polaris* on small and large scallops in choice and nonchoice treatments per replicate during 1994 and 1995 experiments.

were 2.67 for larger scallops and 1.44 ± 0.77 for smaller ones. Statistical analysis showed that larger scallops were consumed more often than smaller ones in both treatments ($p < .05$). In 1995, *C. irroratus* consumed more smaller scallops after 24 hours, 11 ± 0.71 per predator, than larger ones (3.75 ± 1.06) in nonchoice treatments ($p = .001$). However, larger scallops were consumed more quickly in choice treatments, with a predation rate of 5 ± 0.71 as compared to 2.25 ± 2.47 for smaller ones ($p = .001$).

Small scallop mortality was statistically lower with *H. sp.* ($p = .0001$), but larger scallop consumption was similar for both crab species ($p > .05$). *H. sp.* consumed more larger scallops than smaller ones ($p = .014$) in 13 days (Fig. 6). *C. irroratus* had consumed all of the scallops presented at the end of each replicate. However, 1 day after seeding, *C. irroratus* consumed all large scallops available, four per predator. Large scallops were consumed more often than smaller ones, 2.67 ± 1.04 per predator ($p = .002$).

DISCUSSION

In this study, starfish consumed less than one scallop per predator per day. In neither test year did starfish show a clear size-related preference in choice and nonchoice treatments. However, Barbeau and Scheibling (1994a) showed that, in aquaria, *A. vulgaris* of 30 to 150 mm diameter consumed more scallops of 5 to 8.5 mm shell height than those of 10 to 15 mm or 20 to 25 mm in

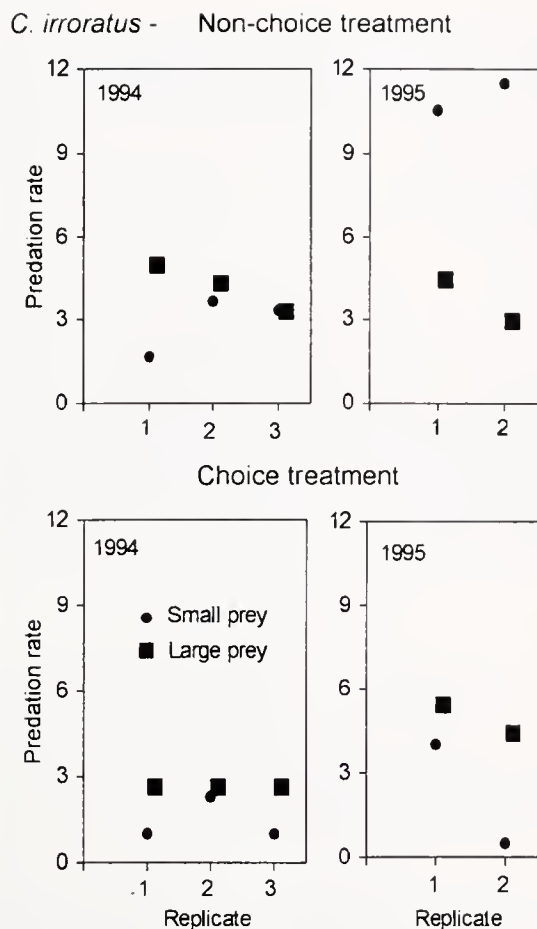


Figure 5. Predation rates (n scallops consumed/predator/24 hours) of *Cancer irroratus* after 1 day on small and large scallops in choice and nonchoice treatments per replicate during 1994 and 1995 experiments.

choice and nonchoice treatments. Under field conditions, Barbeau et al. (1994) confirmed that *A. vulgaris* consumed more scallops of 5 to 9 mm and 10 to 15 mm than 20 to 25 mm. In addition, tests performed by Arsenault and Himmelman (1996) showed that vulnerability of the scallop *Chlamys islandica* (between 10 to 60 mm shell height) to *L. polaris*, *C. papposus*, and *A. vulgaris* decreased with increasing prey size in choice treatments.

Crossaster papposus had lower predation activity (0.05 prey per predator per day) than *A. vulgaris* (0.2 prey per predator per day) on juvenile scallops. However, *A. vulgaris* and *L. polaris* induced comparable mortality rates for juvenile scallops. These last predators spent less than 10% of the recorded time searching for prey. Active scallop escapes and predator retractions, especially involving *L. polaris*, kept the consumption rate under one scallop per predator per day. These findings were confirmed by Barbeau and Scheibling (1994a), in whose tests *A. vulgaris* spent 11 to 27% of time searching for prey, resulting in less than one scallop of 20 to 25 mm being consumed per starfish per day.

Differences between experimental years and replicates could be related to various factors. Differences in years may be partly associated with the experimental design modifications. In 1994, predator density was twice as high and tank sections half as large as in 1995. Thus, active escapes induced after starfish encounters were probably limited by the walls of the smaller tanks used in 1994. The 1995 experimental design more closely simulated natural conditions. Replicates may also have been affected by the

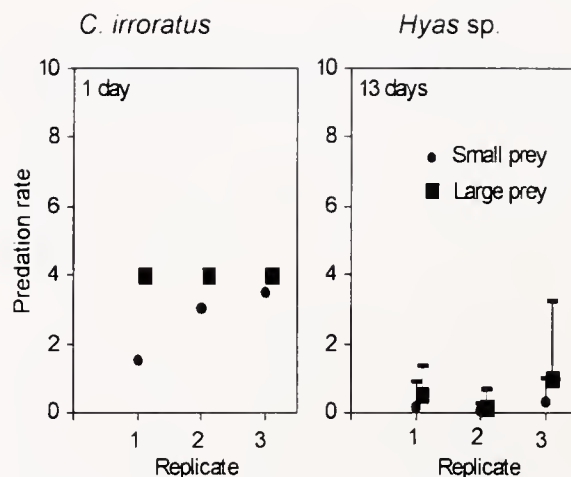


Figure 6. Predation rates (n scallops consumed/predator/24 hours) of *Cancer irroratus* after 1 day and mean predation rates (\pm 95% C.I.) of *Hyas sp.* after 13 days on small and large scallops in choice treatments per replicate during 1995 experiments.

variability in individual behavior and in physiological needs during the year.

Crabs were very effective predators, able to ingest as many as 12 scallops per predator per day. In both experimental years and treatments, *C. irroratus* consumed all scallops available within 24 to 72 hours after seeding. In nonchoice treatments, this crab species tended to consume all available prey rapidly. In choice treatments, the mortality of larger scallops after 24 hours was higher than that of smaller ones. Although *Hyas sp.* also selected larger specimens in choice treatments, the predation rates were lower than those of *C. irroratus*. In fact, the predation rates of *Hyas sp.* (0.5 scallop per predator per day) were closer to the starfish. Barbeau and Scheibling (1994a) showed that *C. irroratus* consumed more scallops of 10 to 15 mm shell height than of 5 to 8.5 mm or 20 to 25 mm in nonchoice treatments, and more scallops of 20 to 25 mm in choice treatments. In contrast, their field testing (Barbeau et al. 1994) on scallops of 7 to 28 mm indicated that prey size had little effect on crab predation success.

Video tapes showed that *C. irroratus* spent 70 to 90% of the time searching for prey. This result may be an overestimate, because all crab displacements were related to searching behavior. Barbeau and Scheibling (1994a) estimated crab searching behavior at less than 11% of the recorded time. In the present study, *C. irroratus* encountered as many as 31 to 66 scallops per day and a reaction of retraction followed in 58.4 to 86.6% of the time. In Barbeau and Scheibling's (1994a) study, no predator retraction was observed, but passive escapes represented $58 \pm 41\%$ of total escapes. However, passive escape was impossible to detect on recording image. Therefore, this escape may have been confused with the retraction behavior. Wilkens (1991) postulated that scallops often respond to crab encounters by closing their valves (passive escape). This behavior may result from scallops detecting crab movement with the use of eyes on the mantle edge.

Elnor and Jamieson (1979) showed that *C. irroratus* had a specific scallop (*P. magellanicus*) size preference in multiple prey choice treatments under controlled conditions. Larger predators (120 to 130 mm carapace width) preferred larger scallops (40 to 50 mm shell height), and smaller predators (90 to 100 mm) tended to prefer smaller scallops (20 to 30 mm). Based on these results, it is possible to hypothesize that the intermediate predator size (100 to

120 mm), used in the present study, would prefer the intermediate scallop size (30 to 40 mm). Lake et al. (1987) also showed that *Cancer pagurus* predation was influenced by predator size and scallop size. The number of scallop *Pecten maximus* (30 to 60 mm shell height) consumed increased with predator size (60 to 140 mm carapace width) and decreased as prey size increased. However, their study did not use scallops smaller than 30 mm. In contrast, Arsenault and Himmelman (1996) showed that *C. irroratus* and *Hyas araneus*, size 112 ± 2 mm (mean \pm SD) carapace width and 97 ± 1 mm carapace length, respectively, preyed more on scallops (*Chlamys islandica*) of 10 to 30 mm shell height than on scallops of 30 to 60 mm in multiple prey choice treatments. Furthermore, the feeding rate of *H. araneus* was about twice that of *C. irroratus*.

Effective predators (*A. vulgaris*, *L. polaris*, *C. papposus*, *C. irroratus*, and *Hyas* sp.) live on the sea bottom, where scallops seeding activities occur in the Îles-de-la-Madeleine. In this study, predation rates may have been biased by the absence of refuge or alternative prey. In addition, interaction between predator species was not evaluated. Nevertheless, we can assume that these predators will cause high mortality in seeded scallops under natural conditions. Based upon our results and those obtained from other studies, bottom seeding should be done, if possible, with scallops greater than the size classes used in this experiment (over 50 mm). On bottoms with high crab density, both size classes of scallops will be vulnerable. Because crabs are highly effective scallop predators, these bottoms must be avoided.

Alternative solutions must be evaluated to minimize the impact of predation. Control of seeding density and predator elimination

(Ventilla 1982) are solutions used in Japan. As observed by Barbeau et al. (1994), predation increases with scallop density and crab density. Barbeau and Scheibling (1994b) also demonstrated that the seeding season may have an important impact on predation, because temperature affects feeding activities of crabs (*C. irroratus*) and starfish (*A. vulgaris*). Seeding at lower temperatures was effective in reducing predation, particularly for starfish. Biochemical analyses of *Pecten maximus* performed by Fleury et al. (1996) showed a very low glucide content in the fall. Lower energy reserves may affect the survival rates of seeded scallops. In addition, stress induced by the seeding operations can reduce their vitality. Fleury et al. (1996) found that seeding stress represented high energy expenditures for scallops (*Pecten maximus*). Finally, weak animals escape predators less often and less rapidly (Hatcher et al. 1996). Seeding strategies will have to deal with all these factors. Seeding operations in the Îles-de-la-Madeleine will be financially profitable if 20 to 30% of the scallops seeded are caught by scallop fisherman. An effort must be made to ensure such survival rates.

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GROWTH, PRODUCTION, AND REPRODUCTION IN BAY SCALLOPS *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY) FROM THE NORTHERN GULF OF MEXICO

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ABSTRACT The bay scallop, *Argopecten irradians*, is a commercially and recreationally important fisheries species on the Atlantic and Gulf Coasts of the United States. Surprisingly, little information exists on northern Gulf of Mexico populations. This research assessed growth, production, and reproduction in a population from St. Joseph Bay, Florida. Specifically, scallops exhibited high initial growth rates ($Gw\ day^{-1}$), with rates declining as individual size increased. Additionally, significant interannual variability existed for both scallop growth and production. These differences may be attributable, in part, to a reduction in salinity (21‰) associated with Tropical Storm Alberto, which resulted in significantly lower growth rates and a mass mortality event. Reproductive assessment of *A. irradians* showed significant peaks in spawning condition, gonad weight, and gonadal-somatic index (GSI) during the winter (December, January, February) compared to other seasons. However, the salinity minima in July 1994 (11‰) significantly reduced gonad weight and GSI from winter 1994/1995, suggesting that a single storm event had a dramatic but short-term reproductive impact on the population. Assessment of gonad condition and GSI, coupled with field observations, showed spawning occurred in the spring and fall as well, but the presence of small individuals (<4 mm shell height) during July, August, and September suggests that reproduction may occur throughout the year in St. Joseph Bay, Florida.

KEY WORDS: *Argopecten irradians*, gonad weight, GSI, Gulf of Mexico, salinity effects

INTRODUCTION

Bay scallops, *Argopecten irradians* (Lamarck 1819) are common members of many shallow water benthic communities along the Atlantic and Gulf Coasts of North America. Clarke (1965) identified three distinct subspecies of *A. irradians* based on morphologic characteristics, and recent morphologic and genetic studies support these distinctions (Wilbur and Gaffney 1997). Although it is recognized that scallops recruit to seagrass habitats (Gutsell 1930, Thayer and Stuart 1974, Eckman 1987) and that they cling to leaves to escape benthic predators (Pohle et al. 1991, Ambrose and Irlandi 1992), little information exists on several key aspects of their ecology. Several studies have assessed scallop growth rates under experimental conditions determining the impact of flow (Kirby-Smith 1972, Cahalan et al. 1989, Eckman et al. 1989) or food concentration and stocking density (Rheault and Rice 1996), but there are few field estimates of adult growth rates under natural, noncaged conditions. Eckman (1987) showed greater growth rates for juvenile bay scallops in grass beds with low shoot densities, and Ambrose and Irlandi (1992) showed that juveniles may trade-off decreased growth rates for increased survivorship by altering their placement on seagrass leaves. In addition, it has been shown that habitat configuration has an impact on the growth and survival of juveniles (Irlandi et al. 1995). Although these studies adequately assess growth of juveniles, few published studies exist on growth rates of adult scallops under natural field conditions.

Another aspect of *A. irradians* is the relative lack of estimates of natural mortality (but see Marshall 1963). Many studies have assessed predation mortality (Tettelbach 1986, Pitcher and Butler 1987, Peterson et al. 1989, Prescott 1990, Pohle et al. 1991, Ambrose and Irlandi 1992, Bologna 1998); however, few estimates of natural population mortality exist. Exceptions include clear patterns of high postspawn mortality (Gutsell 1930, Capuzzo and Hampson 1984), mortality associated with 2nd-year individuals prior to spawning (Bricelj et al. 1987a), and the severe impacts

of nuisance algal blooms (Summerson and Peterson 1990, Tettelbach and Wenzel 1993).

One feature of *A. irradians* ecology that is relatively well known for many populations is reproductive effort. Given that scallops suffer high mortality after spawning, significant research has focused on reproduction, reproductive conditioning, and changes in biomass associated with gonad development (Bricelj et al. 1987b). It has been shown that scallops show distinct peaks in spawning, and these peaks differ temporally based on latitude of the populations (Sastry 1970, Barber and Blake 1983, Crenshaw et al. 1991). Although scallops do show peaks in reproduction, recruiting individuals (<10 mm shell height) have been reported throughout the year, suggesting that trickle spawning may occur in some locations (Gutsell 1930, and see Bricelj et al. 1987b). However, in general, bay scallops are considered to be a semelparous or short-lived iteroparous species, and reproductive effort tends to follow this life history trait (Sastry 1970, Sastry 1979, Barber and Blake 1983, Bricelj et al. 1987b).

Despite a wealth of knowledge on populations of *A. irradians* from the Atlantic Coast and southern Florida, little is known about northern Gulf of Mexico populations. The goals of this research were to assess several life history traits from a bay scallop population from St. Joseph Bay, Florida, U.S.A. Specifically, research focused on the following objectives: (1) determine the natural growth rate of adult scallops; (2) estimate natural population mortality; (3) estimate production within the population; (4) determine reproductive cycles; and (5) assess reproductive effort of scallops.

METHODS

Study Site

This research was conducted in St. Joseph Bay, Florida, which lies in the northeastern Gulf of Mexico (29° N, 85.5° W). It is a semi-enclosed lagoonal system with little freshwater input. Salinity and temperature data were collected from 1992 through 1995 in St. Joseph Bay (Fig. 1) and show that temperature follows a sea-

sonal trend; whereas, salinity is normally high and ranges from 25 to 35‰ annually. However, large storm events (e.g., Tropical Storm Alberto) can significantly reduce salinity over short periods of time (e.g., to 11‰).

Shallow regions of the St. Joseph Bay benthos primarily consist of the seagrass *Thalassia testudinum* interspersed with *Halodule wrightii*, *Syringodium filiforme*, and open sediment. *T. testudinum* is the dominant species and covers approximately 2,300–2,400 hectares (Savastano et al. 1974, Iverson and Bittaker 1986).

GROWTH, MORTALITY, AND PRODUCTION

To determine rates of growth and mortality, a series of mark-recapture experiments were undertaken. Scallops were marked by cleaning and drying the ventral valve and gluing on a numbered tag. Scallop shell height and breadth were then measured using vernier calipers to the closest 0.05 mm. Six additional scallops were marked and held in aquaria for 2 weeks to assess the impacts of handling and marking on survival. All six control individuals survived the 2 week trial and were released back into the field. These individuals were not used to estimate growth, mortality, or production.

In 1993, 79 scallops (31.3 mm to 44.1 mm shell height) were marked and released on two dates in June into an expansive *Thalassia testudinum* grass bed. Scallops were marked and released on June 11 ($n = 31$) and June 14 ($n = 48$). During 1993, scallops were relocated and measured on June 14, 23, July 8, 22, August 25, September 19, November 5, and December 11. In 1994, 95 scallops were marked in three cohorts. In April, 40 scallops (37.75–66.3 mm shell height) were marked and measured in the same manner as above. The second cohort of 33 scallops (41–51.6 mm shell height) was marked on June 23, and the final scallop cohort ($n = 22$, 41.8–53.8 mm shell height) was marked and released on July 14. All cohorts were released into an extensive *T. testudinum* grass bed mosaic and periodically sampled throughout the year. Specifically, scallops were relocated on May 5, June 6, 23, July 7, 14, 20, August 10, 18, 31, and October 12.

Estimates of growth and mortality followed the protocol of Cowan and Houde (1990). Instantaneous growth rate was calculated using the change in biomass over time. Scallop biomass was estimated using the following regression equation from Bologna (1998), which provides an estimate of tissue dry weight: ($\ln[\text{tissue}$

dry wt. (g)] = $-9.779 + 0.7909 \ln[\text{shell height}] + 2.2124 \ln[\text{shell breadth}]$; $n = 161$, $r^2 = .92$). Growth rate (\hat{G}_w , growth in weight), expressed as day^{-1} , was computed using the Eq. 1.

$$\hat{G}_w = \frac{\ln(W_t) - \ln(W_{t-1})}{t} \quad (1)$$

where: \hat{G}_w = instantaneous growth rate (day^{-1}); W = estimated scallop dry weight at time t ($\ln[\text{grams tissue dry weight}]$); and, t = time in days. Instantaneous scallop mortality rate (μ) was estimated from recaptures using Eq. 2.

$$\mu = \frac{\ln(n_0) - \ln(nt)}{t} \quad (2)$$

where: μ = calculated instantaneous mortality rate (day^{-1}); n = is the number of scallops present at a given time t ; and t = time.

Because scallops were motile (>500 m, pers. obs., this study), calculated mortality actually reflects mortality plus emigration and, consequently, is an overestimate. To limit the impact of initial emigration bias on mortality estimates by second and third cohort marked scallops (e.g., field release dates June 14, 1993, June 23 and July 14, 1994), mortality on successive dates (June 23, 1993, July 7 and 20, 1994) was calculated using the number of scallops present from the previous cohort. Subsequent mortality estimates were based on any individual present beyond one sampling date.

Based on estimated instantaneous growth and mortality rates calculated from mark-recapture scallops, production (g dry wt day^{-1}) for time intervals was calculated using the Cowan and Houde (1990) equation:

$$\hat{P} = \bar{B} * \hat{G}_w \quad (3)$$

where: \bar{B} = average biomass (g) during a time interval; \hat{G}_w = instantaneous growth rate; and \bar{B} was calculated as follows.

$$\bar{B} = \frac{B_0 [e^{(\hat{G}_w - \mu)t} - 1]}{(\hat{G}_w - \mu)t} \text{ for } (\hat{G}_w > \mu)$$

$$\bar{B} = \frac{B_0 [1 - e^{-(\mu - \hat{G}_w)t}]}{(\mu - \hat{G}_w)t} \text{ for } (\mu > \hat{G}_w) \quad (4)$$

where: B_0 = estimated initial biomass of all marked individuals for a given time interval; \hat{G}_w = instantaneous growth rate; μ = instantaneous mortality rate; and, t = time.

Growth, mortality, and production rates were compared between 1993 and 1994 using a nonparametric two-tail trend analysis ($\alpha = 0.05$) for rates on similar calendar dates between years.

REPRODUCTION

Scallop reproduction was assessed by visual inspection of the gonad condition and resultant gonad weight and relative weight ratio (GSI). Scallops were collected on 37 dates from St. Joseph Bay, Florida from November 1992 to October 1996. Scallops were frozen and returned to the laboratory where their shell height and breadth were measured to 0.05 mm. Scallops <22 mm were considered juveniles, and body tissue was dissected and weighed as a whole. Scallops >22 mm shell height had both gonadal and somatic tissue dissected out.

Reproductive and somatic tissue were then dried at 80°C for 48 to 72 hours and weighed (g dry weight). A GSI was calculated for each scallop using the following equation: $\text{GSI} = (\text{gonad dry weight}/\text{total dry weight}) * 100$. To assess seasonal timing of maxi-

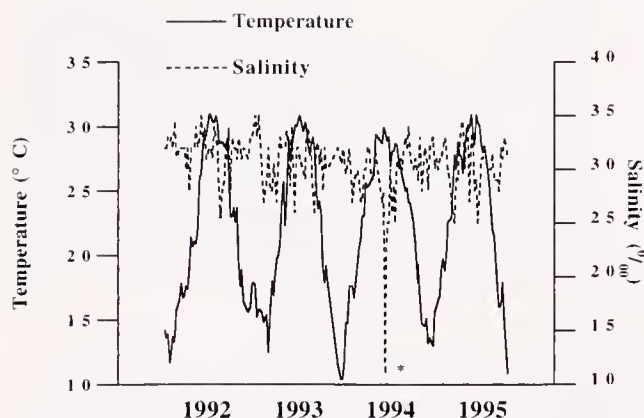


Figure 1. Weekly temperature ($^\circ\text{C}$) and salinity (‰) values collected from St. Joseph Bay, Florida from January 1992 through December 1995. Source: City of Port St. Joe, Florida industrial waste-water treatment plant, 1996; * indicates a salinity minima of 11‰ on July 27, 1994.

mal GSI and gonad weight, collections were groups as winter (samples collected in December, January, and February), spring (March, April, and May), summer (June, July, August) and fall (September, October, November). Data were analyzed using a one-way analysis of variance (ANOVA; $\alpha = 0.05$), with season as the independent variable and gonad weight and GSI as dependent variables. Multiple comparisons were made using Scheffe's F-test. In addition, scallop gonad weight was analyzed against date of collection using one-way ANOVA to determine annual variability in reproductive effort.

Visual condition of gonadal material for scallops greater than 22 mm shell height was assessed for each individual beginning in August 1993. Gonad condition was assessed as undeveloped, ripening, very ripe, or postspawn. Undeveloped gonads appeared gray in color, but were robust in nature. Ripening gonads were robust and showed visual development of gonadal material (i.e., female portions were, to some degree, red-orange in color and/or male portions were white or whitening). Very ripe gonads were robust, with female portions fully red-orange and male portions entirely white. Gonads from postspawn individuals were either gray and flaccid or gray with tinges of red-orange/white and flaccid (as opposed to robust, which were associated with undeveloped and developing gonads).

Evidence of reproduction was assessed by collection of small individuals (<20 mm shell height). This was accomplished by visually locating them in the field and returning them to the laboratory for morphological measurement and biomass determination. In addition, in 1994 and 1995, artificial seagrass mats (for a full description of seagrass mat construction and characteristics, see Bologna 1998) were placed in the field during the summer to determine the presence and recruitment density of bay scallops.

RESULTS

Growth, Mortality, and Production

Scallop growth, mortality, and production rates are summarized in Table 1. In 1993, scallops showed growth rates that were relatively high, declining slowly from June to September and dramatically in the fall. However, scallops marked in 1994 showed a rapid decline in growth rate from May to June and maintained very low growth rates throughout the summer and into the fall. When the data were analyzed using trend analysis, calculated compared mean scallop growth rate for 1993 was significantly greater than growth rates in 1994 ($P[K \leq 0.17, 0.475] = 0.0117$).

Scallop mortality rates were initially relatively high for all series of mark-recapture scallops (Table 1). This may be attributed to relatively high emigration (dispersal) rates after initial deployment into the field, and these mortality estimates must be treated with caution. The calculated mortality rates for scallops on subsequent cohort release dates in 1993 and 1994 did not show this, because mortality calculations were based on previous individuals in the field and not the potentially high rates of emigration on initial placement in the field. In 1993, mortality was high during June, July, and August, but relatively reduced in September–November, with major losses by the December collection date. No mortality patterns were present in 1994 data. However, mortality rates were lower in 1994 than 1993 (Table 1), albeit not significant ($P[K < 3.17, 0.475] = 0.574$). However, the relatively high mortality rate calculated for August 10, 1994 corresponds to the salinity minima of 11‰ seen in 1994 from freshwater input associated with Tropical Storm Alberto (Fig. 1).

TABLE 1.
Instantaneous growth, mortality, and production rates for mark-recapture *A. irradians* from 1993 and 1994.

Date	n	cd	Growth Day ⁻¹	Mortality Day ⁻¹	Production g Day ⁻¹
6/14/93*	24	a	0.02509	0.07380	0.33560
6/23/93	54 (19)	b	0.01865	0.02696	0.68474
7/8/93	38	c	0.02027	0.02343	0.49523
7/22/93	26	d	0.01638	0.02711	0.48080
8/25/93	15	e	0.01054	0.01618	0.27561
9/19/93	13	f	0.01064	0.00572	0.33591
11/5/93	9	g	0.00539	0.00782	0.11650
12/11/93	2		0.00224	0.04178	0.01852
1994					
5/5/94	21		0.02603	0.03391	0.5998
6/9/94	14	a	0.00544	0.01158	0.1722
6/23/94**	11	b	0.00417	0.01723	0.1049
7/7/94	19 (9)	c	0.00431	0.01433	0.0905
7/14/94***	18		0.00632	0.00772	0.1889
7/20/94	25 (17)	d	0.00442	0.00817	0.1238
8/10/94	17		0.00469	0.01836	0.1589
8/18/94	16	e	0.00650	0.00758	0.1743
8/31/94	14	f	0.00638	0.01027	0.1627
10/12/94	5	g	0.00343	0.02451	0.0584

Growth and mortality values expressed as day⁻¹, production expressed as grams day⁻¹; n indicates the number of recaptured individuals used to estimate growth, mortality, and production; values in parentheses indicate the number of individuals used to estimate mortality when a successive cohort of marked individuals was added to the field, thus limiting initial emigration bias of mortality by newly placed marked scallops; cd indicates calendar dates of comparison for growth, mortality, and production rates between 1993 and 1994; * indicates the date when 48 additional individuals were marked and released into the field; ** indicates the addition of 33 marked scallops; *** indicates the addition of 22 marked scallops.

Scallop production in 1993 showed an increase during the initial part of the study with declining productivity as the year progressed (Table 1). Because production was based upon estimates of mortality that include both mortality and emigration, calculated production is a conservative estimate. In 1994, production was considerably lower and remained less than one-third that of 1993. Results from trend analysis showed a significant reduction in scallop production from 1993 compared to 1994 ($P[K < 0.17, 0.475] = 0.0117$). This reduction in production between years was likely the result of the significantly lower growth rates in 1994 compared to 1993 ($p < .023$); and not to changes in relative mortality (Table 1).

A scallop mortality event was observed on July 20, 1994 throughout the southern half of St. Joseph Bay. Although the absolute salinity minima (11‰) occurred on July 27 (see Fig. 1), the reduction in salinity attributable to Tropical Storm Alberto on July 3, not to mention the potential for disturbance and burial, may have had a severe impact on the survival of bay scallops. The observed salinity minima, however, corresponded well to the high estimated instantaneous mortality rate calculated for mark-recapture individuals on the following collection date (August 10, 1994, Table 1). On June 24, 1996, another mortality event was observed in St. Joseph Bay. During this time period, numerous sitings of red-tide algal blooms occurred along the coastal regions of the northeast Gulf of Mexico (J. Stout, pers. comm.) and may have been responsible for this mortality event.

REPRODUCTION

Reproduction assessment by visual inspection of the gonad condition showed several interesting features regarding reproductive individuals (Table 2). First, scallops as small as 31 mm shell height showed very ripe gonads and scallops as small as 34.1 mm shell height showed gonad condition indicative of postspawn (May 1994, Table 2). Second, scallop gonad condition associated with individuals from the winter of 1994/1995 showed relatively low proportions of individuals in any reproductive condition, as well as few individuals (one of 59) showing a postspawn condition (Table 2). Last, of the 1,106 scallops assessed for reproductive purposes, only one individual (October 1995, Table 2) showed nonhermaphroditic sex development with only male gonadal tissue present.

When scallop reproduction was assessed using GSI, results showed scallops had significantly greater GSI values during the

TABLE 2.
Visual gonad condition index.

Date	n	Juvenile	Undeveloped	Ripe	Very Ripe	Postspawn
8/25/93	12	0.00	66.67	33.33	0.00	0.00
9/11/93	27	0.00	96.30	3.70	0.00	0.00
9/19/93	27	0.00	37.04	59.26	3.70	0.00
10/1/93	37	0.00	45.95	40.54	13.51	0.00
11/5/93	40	0.00	47.50	0.00	52.50	0.00
11/19/93	24	0.00	16.67	41.67	25.00	16.67
12/11/93	32	0.00	15.63	0.00	50.00	34.38
1/9/94	36	0.00	11.11	11.11	63.89	13.89
2/25/94	22	0.00	4.55	63.64	27.27	4.55
4/15/94	29	0.00	44.83	24.14	13.79	17.24
5/5/94	45	0.00	2.22	60.00	24.44*	13.33**
6/8/94	44	0.00	95.45	0.00	0.00	4.55
7/20/94	23	0.00	52.17	0.00	0.00	47.83
8/19/94	45	0.00	86.67	8.89	0.00	4.44
8/24/94	45	0.00	42.22	35.56	2.22	20.00
12/1/94	6	0.00	66.67	0.00	33.33	0.00
1/21/95	25	28.00	44.00	20.00	4.00	4.00
2/1/95	28	17.86	57.14	21.43	3.57	0.00
3/10/95	28	10.71	71.43	10.71	7.14	0.00
4/9/95	37	2.70	72.97	13.51	10.81	0.00
5/17/95	42	0.00	83.33	7.14	9.52	0.00
6/20/95	34	0.00	100.00	0.00	0.00	0.00
7/5/95	31	0.00	100.00	0.00	0.00	0.00
8/6/95	32	0.00	68.75	15.63	15.63	0.00
8/30/95	17	0.00	47.06	52.94	0.00	0.00
9/26/95	37	43.24	45.95	10.81	0.00	0.00
10/28/95	22	0.00	0.00	9.09	90.91†	0.00
12/5/95	6	0.00	0.00	50.00	33.33	16.67
1/20/96	13	23.08	0.00	7.69	69.23	0.00
2/15/96	20	0.00	5.00	0.00	95.00	0.00
3/13/96	28	42.86	10.71	10.71	35.71	0.00
4/19/96	24	8.33	62.50	12.50	16.67	0.00
5/22/96	21	0.00	52.38	0.00	4.76	42.86
6/24/96	21	0.00	100.00	0.00	0.00	0.00
8/8/96	16	0.00	68.75	31.25	0.00	0.00
10/18/96	19	0.00	5.26	21.05	73.68	0.00

Values expressed as percentage of sample classified in each category; n indicates the number of scallops collected for reproductive assessment; * indicates a scallop with 31 mm shell height exhibiting this condition; ** indicates a scallop with 34.1 mm shell height exhibiting this condition; † indicates a scallop with only male gonadal development.

winter as compared to other seasons ($F_{3,989} = 241.2$, $p < .0001$, Fig. 2). In addition, scallop gonad weight was significantly greater for samples collected during the winter as compared to other seasons ($F_{3,989} = 107.6$, $p < .0001$). However, both GSI and gonad weight varied significantly among dates among years ($F_{35,957} = 57.5$, $p < .0001$; $F_{35,957} = 47.7$, $p < .0001$, respectively). Specifically, significant reductions in monthly means occurred in both GSI and gonad weight during the 1994/1995 winter (Fig. 3), which corresponds well to the poor gonad development seen in the gonad condition index above (Table 2). These reductions in GSI and gonad weight may have been a result of osmotic stress associated with the salinity minima during 1994 (Fig. 1).

A clear peak in scallop reproduction existed during the winter, with mean GSI values exceeding 15% dry tissue weight (Fig. 2). However, based on gonad condition and GSI, significant reproductive effort also occurred in the fall and spring (Table 2, Fig. 3). On May 17, 1995 a bay scallop mass spawning event was observed. This event occurred while collecting individuals for reproductive assessment and natural population surveys. Specifically, scallops held in a catch-bag ($n \sim 50-75$) spontaneously released gametes. Consequently, independent of GSI, this observation showed that scallops do spawn in late spring.

Support of year-long reproductive effort and success was assessed by the presence of small scallops (<20 mm shell height) collected in the field. Based on these data, it seems that scallops showed some signs of recruitment throughout the year (Table 3). In addition, during the summer of 1994, a companion study assessing the effects of seagrass habitat architecture on bivalve recruitment collected recruiting bay scallops (0.5–2 mm shell height) on novel substrata and from *Thalassia testudinum* (turtle grass). These data showed that scallops were recruiting to novel substrata at densities of 8.09, 4.33, and 6.07 individuals m^{-2} for the months of July, August, and September, respectively. Based on the length of larval development (10–14 days, Sastry 1965, Lu and Blake 1997), these periods of recruitment correspond to potential spawning events during June, July, and August, when minimum values in both gonad weight and GSI existed (Fig. 3).

DISCUSSION

For species of economic value assessing growth, production, and reproduction in populations are essential for both wise man-

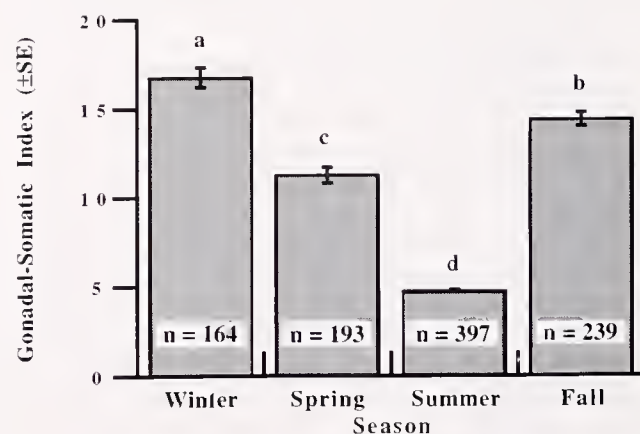


Figure 2. Comparison of mean scallop GSI for seasons pooled across years; n = the number of scallops collected upon which the means are based; letters above seasons indicate significant differences among means in GSI ($\alpha = 0.05$).

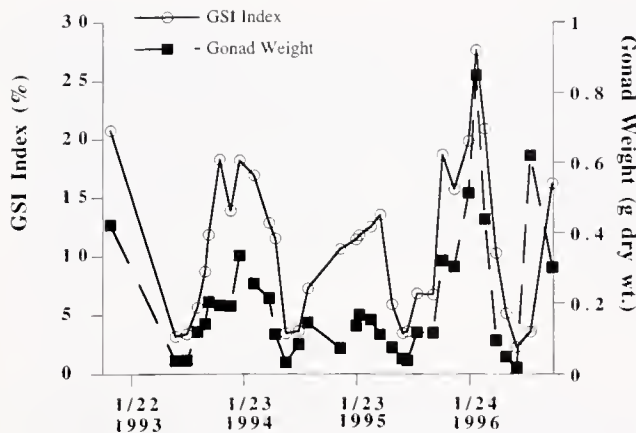


Figure 3. Seasonal patterns in scallop GSI and gonad weight from individuals collected between November 1992 through October 1996. Peaks in GSI and gonad weight occurred during the winter, and minimum values were recorded during the summer. Significant interannual variability in gonad weight occurred and was significantly reduced during the winter of 1994/1995.

agement of healthy populations and conservation and enhancement of endangered populations. Results from this research have shown that bay scallops from St. Joseph Bay, Florida exhibit high growth rates throughout the summer but have dramatic declines in the fall (Table 1). This observation of differential growth as body size increases has also been shown for larvae and juveniles (Lu and Blake 1996) and was similar to results found by Barber and Blake (1983) for scallops collected from the eastern Gulf of Mexico. This seasonal trend was evident in both 1993 and 1994, but in 1994, reduction in salinity by 21‰ may have had a significant impact on the natural growth rates of scallops. Although other researchers have shown that salinity has an impact on the survival of scallops (Duggan 1975, Mercaldo and Rhodes 1982), this is the first case where coupled measurements of physical parameters can be applied directly to differential natural growth rates.

Similarly, estimates of natural "mortality" (mortality plus emi-

TABLE 3.

Presence of recruiting scallops (<20 mm shell height) during 1994 to 1996.

Month	1994	1995	1996
January		*	*
February		*	
March	†	*	*
April		*	*
May		*	
June			
July	*+		†
August	*+	*+	
September	*+		†
October	†		
November	†	†	NA
December			NA

Presence denoted by *; *+ indicates recruiting individuals (<4 mm shell height) collected from suction dredge samples; † indicates samples were not collected during this month; NA indicates the termination of scallop collection.

gration) from field studies ranged from 0.005 to 0.025 for non-placement periods (Table 1) and were similar to those of Allison and Brand (1995), who used a similar mark-recapture technique on *Aequipecten opercularis*. In addition, the rapid dispersal after initial deployment has been recorded from other studies (Barbeau et al. 1996, Hatcher et al. 1996). However, the dramatic mass mortality seen on July 20, 1994 in the field suggests a direct correlation between scallop mortality and the decline in salinity associated with Tropical Storm Alberto (Fig. 1). The observed mass mortality in the field was also seen in the instantaneous mortality for mark-recapture scallops but correlates better with the salinity minima (Fig. 1, Table 1) and provides experimental evidence to the observed pattern of high mortality. Based on Mercaldo and Rhodes (1982) estimates, the drop in salinity to 11‰ during July 1994 could have created conditions such that less than 20% of the scallop population within St. Joseph Bay, Florida may have survived. This corresponds well with this author's inability to locate scallops for reproductive assessment during the fall of 1994 in St. Joseph Bay and reported low abundances by Arnold et al. (1998) for scallop surveys for the same time period.

The impact of reduced salinity on mortality may have had dramatic impacts on the population as a whole. Results from reproductive assessment of the population showed a significant reduction in GSI and gonad weight during the following winter of 1994/1995 (Fig. 3). Because reproductive output is directly associated with gonad development (Sastri 1970), the reduction in gonad weight during the winter of 1994/1995 and the relative lack of gonad development seen in the condition index (Table 2) suggests a limited reproductive output during this season. Given the short life span of *A. irradians* (Guttsell 1930) and significant increases in GSI and gonad weight during the winter of 1995/1996, it seems that no multiple year impacts were evident and corresponds to Barber and Blake's (1986) suggestion that west Florida populations may be stable given their relatively high reproductive effort. In fact, both scallop GSI and gonad weights were the greatest during the fall of 1995 and winter 1995/1996 suggesting a full recovery of the population, despite losses during the previous year.

By assessing the reproductive potential of the population using a gonad condition index, the data suggest that at least some members of the population show reproductive output throughout the year (i.e., very ripe, postspawn, Table 2), and this response has been observed for other scallop species as well (O'Connor and Heasman 1996). Data clearly show peaks in reproduction for St. Joseph Bay scallops occurring in the winter (Figs. 2, 3), later than that of other southern Florida populations (Barber and Blake 1983, Arnold et al. 1998) and approximately opposite those of Atlantic populations (Guttsell 1930, Sastri 1970, Bricelj et al. 1987b, Peterson et al. 1996). In addition, the observation of the spawning event on May 17, 1995 may correlate with increasing water temperatures (Fig. 1), which seem to induce the Atlantic population to spawn (Sastri 1963, Sastri 1970, Bricelj et al. 1987b) and gives a proximal mechanism to explain the multiple spawning seen in St. Joseph Bay, Florida. This study differs from others that have assessed reproduction, because presence of juveniles was also assessed throughout the year. Consequently, results suggest that this population exhibits year-long reproduction, as evidenced by juvenile recruits (Table 3), even when mean GSI and gonad weight were at their minimum values during the year (Fig. 3).

The mass mortality observed in the field on June 24, 1996, however, has few direct physical correlates (e.g., salinity). Red tide

was observed in the northeastern Gulf of Mexico during this time period (J. Stout, pers. comm.), but no clear documentation of the event currently exists. However, Tester and Steidinger (1997) have shown that red tide (*Gymnodinium breve*) is a common member of the phytoplankton communities in the Gulf of Mexico and is present all year. In addition, Tettelbach and Wenzel (1993) and Smolowitz and Shumway (1997) have shown dramatic toxic effects of nuisance algal blooms on both juvenile and adult survival and growth. Therefore, it might be possible that the mass die-off observed in the field on June 24 may have been a result of a local red tide event.

In summary, scallops from St. Joseph Bay showed significant seasonal and annual variability in growth and reproduction. Specifically, the effects of reduced salinity associated with Tropical Storm Alberto had significant impacts on the population through direct mortality and reduced gonadal tissue development. However, these probably only affected a single cohort of *A. irradians*, because the population seemed to recover during 1995 and 1996, and data showed a significant increase in reproductive output. Unfortunately, few studies have assessed bay scallop production. Perhaps this is because of the relatively short life span of *A. irradians* or the lack of mark-recapture estimated field growth and mortality rates from natural populations. Results from this research indicate significant annual variation, probably mediated by physi-

cal parameters, and this information may provide a basis for future comparisons among bay scallop populations. In addition, the presence of recruiting scallops throughout most months of the year and the identification of a significant spawning event, suggests that future assessments of the reproductive output and population structure of Gulf of Mexico bay scallops target the entire year and not only winter peaks in reproduction. Last, the identification of small scallops involved in reproduction (<35 mm shell height) suggests that future research should also address the potential these individuals have on the reproductive output and success of populations.

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GONADAL MATURATION, CONDITIONING, AND SPAWNING IN THE LABORATORY AND MATURATION CYCLE IN THE WILD OF *CERASTODERMA GLAUCUM* BRUGUIÈRE

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ABSTRACT *Cerastoderma glaucum* Bruguière has a wide geographical distribution in Europe, from the Mediterranean to the North Atlantic and the Baltic Sea. This cockle is found mainly in coastal embayments and lagoons on muddy, soft bottom. The different reproductive behavior of cockles is related to latitude. In Lesina Lagoon, cockles initiate gametogenesis several times per year; this has been confirmed in laboratory tests where cockles are provided ample Tahitian *Isochrysis* aff. *galbana* (clone T-ISO). In the laboratory, cockle biomass (density 400–800 g/m²) releases viable gametes all year in a relatively wide range of temperatures (9–24°C). During 12 months of repeated spawning, cockles released a mean of 68,100 eggs/cockle, with an average of 1.13 spawning per week. Because of its high genetic variability, which accounts for its adaptability and ease of spreading in environments with striking changes of physical and chemical aquatic conditions, cockles may have an excellent potential for mariculture application. In addition cockles may play an important ecological role in reducing the particulate organic load of eutrophic lagoons and embayments with a wide range type of salinity and thermal characteristics.

KEY WORDS: cockle, bivalve, mollusc, spawning, conditioning

INTRODUCTION

Bivalve mollusks derived from fisheries and farming are a considerable part of the total sea food landed (30% of the world aquaculture, Manzi and Castagna 1989). With the success of worldwide mussel cultivation, research efforts are underway to match the production of clams, oysters, and scallops. Indeed, during the last two decades, scientists have successfully reared more than 20 new bivalve species (Manzi and Castagna 1989). Despite such success, the scientific community is being asked to propagate potential new species for farming and/or restocking overexploited areas. In addition to the edible aspect of farmed organisms, bivalve molluscs are important as filter feeders for the removal of particulate organic matter load of eutrophic lagoons and embayments (Glude 1984, Mann and Ryther 1977). One candidate bivalve to this purpose is the dioecious cockle *Cerastoderma glaucum* Bruguière. This cockle commonly occurs from the Baltic and North Seas to the Mediterranean Sea (Boyden and Russell 1972, Zaouali 1975, Labourg and Lasserre 1980, Brock and Christiansen 1989) (Fig. 1). *C. glaucum* preferentially dwells on muddy bottoms of lagoons and estuaries. Because of their wide genetic variability (Brock and Christiansen 1989), cockles occur in frontier areas where the environmental conditions fluctuate at the extremes (Rygg 1970, Zaouali 1975, Labourg and Lasserre 1980). This makes *C. glaucum* an interesting subject for farming and/or reducing the environmental impact of organic loading in estuarine systems. This study examines the maturation conditioning and spawning of cockles in relation to water temperature and diet regimes.

MATERIALS AND METHODS

This study examines the annual gametogenic cycle of *C. glaucum* from Lesina Lagoon (Fig. 2). In addition some *C. glaucum* specimens were tested in the laboratory for maturation conditioning and successive spawning. Laboratory trials were carried out either in 40- to 600-L tanks. These vessels were supplied with lagoon water after filtering through a 1- μ m pore size cartridge. The residence time of the water in the 40- and 600-L tanks was 6 and 48 hours, respectively. For the conditioning and spawning tests, field-collected cockles with minimum average size of 4 g

were stocked in 40-L tanks with biomass density ranging from 400 and 800 g/m². They were fed with unicellular algae culture of Tahitian *Isochrysis* aff. *galbana* Green (clone T-ISO), *Dunaliella tertiolecta* (Butcher), and *Tetraselmis suecica* Kylin (Butcher), grown in the artificial light (Trotta 1981) and fed to cockles over 24 hours at the rate of 2.5 to 5% (dw/dw) per day. Photoperiod was set at 12 light/12 dark. Incoming water salinity ranged from 18 ppt to 38 ppt.

The maturation phase of the gonads were recorded according to the description by Boyden (1971) and Wolowicz (1987).

- *stage I.* undifferentiated; beginning of gametogenesis; gonads are not developed and sex is not yet distinguishable;
- *stage II.* developmental; gonads start to develop in the foot tissue and around the digestive gland; sex is distinguishable;
- *stage III.* gonads developed; made of a compact tissue; white cream streamed line in the male, yellow cream granular isles in the female;
- *stage IV.* reproductive stage; inflated and ripe gonads; isles in the females and stream line in the males; flowing;
- *stage V.* rest; this is the postreproductive phase or regressive phase; the visceral mass becomes flaccid; gonads are fallen in, often with a small number of remaining gametes, which are distinguishable with difficulty because of the presence of some corpi lutei.

Experiment 1. Maturation Conditioning of *C. Glaucum* Fed T-ISO and *D. Tertiolecta*

Fifty cockles collected at the Fortore River outlet (average size 4.05 g \pm 0.96 (2s)—95% confidence intervals—equivalent to 417 g/m²) were assigned to two 40-L tanks. One tank received a culture of T-ISO and the other a culture of *D. tertiolecta* at a daily rate of 2.5 to 5% (dw/dw). Cockle mean weight in grams was taken at the start and at 30 and 60 days into the experiment. Twenty-five specimens left after the random selection were not fed and were considered as blank (control group in a 40-L tank). At regular intervals, cockles were biopsied to determine gametogenic state. At the end of the experiment, 20 cockles per treatment were taken to check the gonadal stage of maturation.



Figure 1. The reported occurrence of *C. glaucum* in Europe.

Experiment 2. Maturation Conditioning of *C. glaucum* fed T-ISO and *T. suecica*

A second step of the maturation conditioning started with cockles at the stage I (undifferentiated) and gonadal development. This experiment was conducted to confirm the previous test. Sixty cockles sampled from the Varano Lagoon (average size $4.54 \text{ g} \pm 2.6$ equivalent to 542 g/m^2) were assigned to two 40-L tanks and fed, respectively, T-ISO and *T. suecica* at a daily rate of 2.5 to 5% (dw/dw).

Experiment 3. Spawning of Cockles Fed T-ISO and 50/50 Mixture of T-ISO/*T. suecica*, but Previously Conditioned to Maturation in a 600-L tank and Fed T-ISO

After the confirmation of the maturation conditioning from the previous experiment, 500 g of cockles, sampled from the Lesina Lagoon, corresponding to 782 g/m^2 , was put in a 600-L tank for over 2 months and fed T-ISO at the rate of 1% (dw/dw). Cockles were assigned to two 40-L tanks (average size $7.68 \text{ g} \pm 3.94$ corresponding to 800 g/m^2) at 26 specimens per each vessel. Cockles received T-ISO and a 50/50 mixture of T-ISO/*T. suecica*, respectively, at a daily rate of 2.5 to 5% (dw/dw).

After each spontaneous spawn, eggs were collected on a 80- μm sieve after drawing the 40-L tank. Eggs were placed in a graduated cylindrical vessel with gentle aeration. A 2.5-mL subsample was counted for number of eggs with a 2.5-mL inverted microscope

chamber. The counts were validated with the χ^2 test ($\alpha = 0.05$). The laid eggs were assigned to a four classes ranked as follows:

- first-class interval (1) up to 150,000 eggs/tank;
- second-class interval (2) from 150,000 to 300,000 eggs/tank;
- third-class interval (3) from 300,000 to 600,000 eggs/tank; and
- fourth-class interval (4) more than 600,000 eggs/tank.

***C. glaucum* sampled in the wild**

C. glaucum were collected periodically in the Lesina Lagoon as well as at the border sites from 1995 to 1996. Collecting in these latter sites was made necessary when cockles from Lesina Lagoon were lacking.

RESULTS

Experiment 1. Maturation Conditioning of *C. glaucum* Fed T-ISO and *D. tertiolecta*

T-test showed that cockles fed T-ISO (t68 10.1 $\alpha = 0.05$) increased in live weight from 4.05 to 5.37 g in 60 days as compared to a slight increase of those fed *D. tertiolecta* (t68 2.164 $\alpha = 0.05$) from 4.05 to 4.31 g (Table 1). The difference of weight gain in cockles fed T-ISO and *D. tertiolecta* on day 60 was significant (t38 7.515 $\alpha = 0.05$) (Table 1).

The specimens fed T-ISO resulted in 100% of cockles with ripe gonads (stage III and IV) and a sex ratio of 12F:8M₍₁₎; however, only 45% of the cockles fed *D. tertiolecta* had recognizable gonads. Most of these latter were in the regression stage (V). The sex ratio for cockles fed *D. tertiolecta* was 5F:4M₍₁₎, with 11 cockles being undifferentiated. For cockles fed T-ISO, the foot appeared fleshy, yellow-orange colored, and the digestive gland showed large ocher brown acini; whereas, those fed *D. tertiolecta* had flat pale yellow foot and small pale brown acini. The starved cockles (control group), at the end of the experiment, showed developed gonads (stage II and III), with a sex ratio of 9F:9M:1U₍₁₎, but reduced size of foot and pale colored digestive gland. (F = female, M = male, U = undifferentiated)

Experiment 2. Maturation Conditioning of *C. glaucum* Fed T-ISO and *T. suecica*

Field cockles collected for this study were all in the undifferentiated stage (I). After 2 months on the microalgae diets, cockles commenced spawning (Fig. 3), which lasted for 3 months. By midMarch, the surviving specimens (23% T-ISO, 33% *T. suecica*)

TABLE 1.

Average live weight of cockles in grams fed with T-ISO and *D. tertiolecta*

	Average Weight in g	Standard Deviation s
T-ISO		
Start	4.05	0.48
30 Days	4.62	0.65
60 Days	5.37	0.52
<i>D. tertiolecta</i>		
Start	4.05	0.48
30 Days	4.25	0.42
60 Days	4.31	0.36

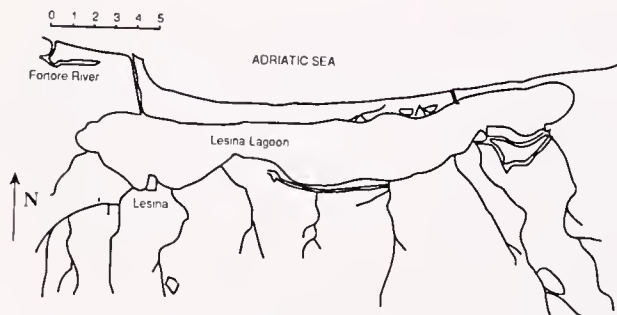


Figure 2. *C. glaucum* used in this study were collected from Lesina Lagoon, Italy.

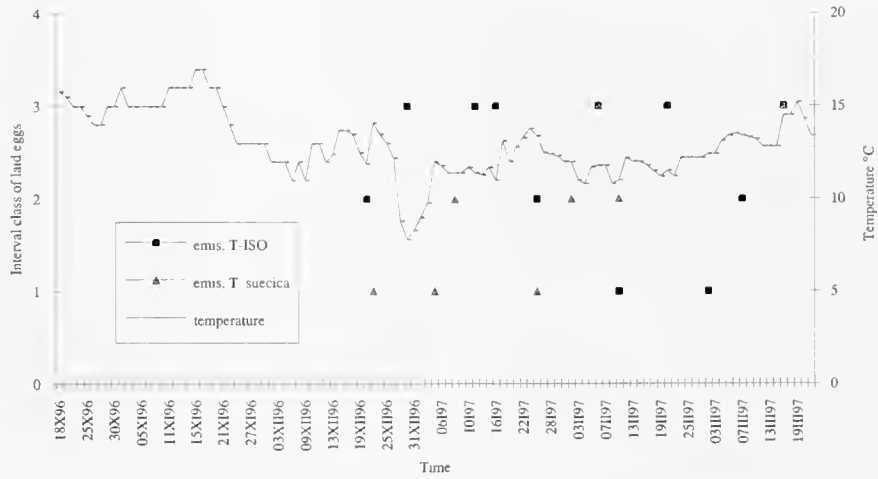


Figure 3. Egg emission from *C. glaucum* broodstock fed either T-ISO or *T. suecica* and related water temperature.

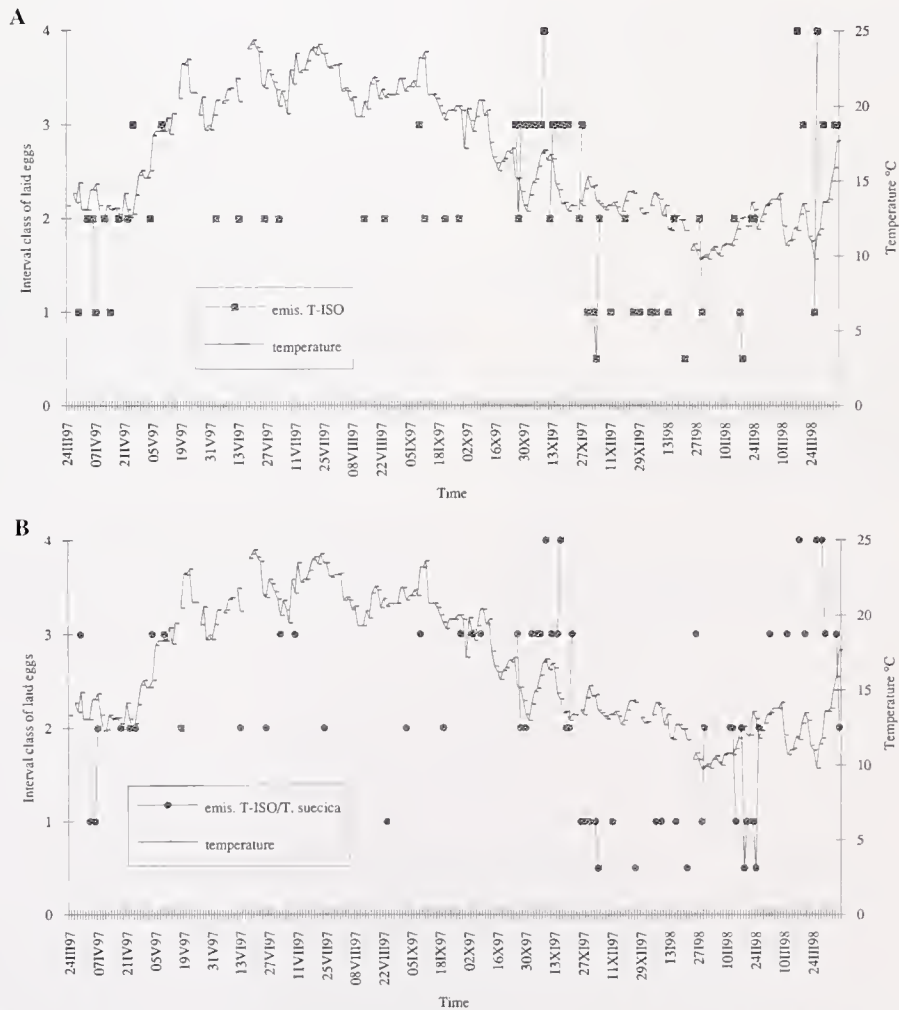


Figure 4A. Egg emission from *C. glaucum* broodstock fed T-ISO and related water temperature. Figure 4B. Egg emission from *C. glaucum* broodstock fed a 50:50 mixture of T-ISO and *T. suecica* and related water temperature.

TABLE 2.

Number of emission and total eggs per year laid by cockles fed either T-ISO and a 50:50 mixture of T-ISO/T. *suecica* in Experiment 3

	T-ISO	T-ISO/T. <i>suecica</i>
Class interval 4	3	3
Class interval 3	17	20
Class interval 2	25	20
Class interval 1	14	15
Total emis./year	59	58
No emis./week	1.13	1.12
No eggs/specimen/year	681,000	687,000

had ripe gonads (stage III and IV), although those fed the latter microalga appeared undernourished after the biopsy test.

Experiment 3. Spawning of Cockles Fed T-ISO and 50/50 Mixture of T-ISO/T. suecica, and Previously Conditioned for 2 Months to Maturation in a 600-L tank with a T-ISO Feeding Regime

The cockles conditioned in a 600-L tank started to spawn immediately after stocking in the 40-L tanks. The emission of the gametes occurred for 12 months, during which the water temperature regime ranged from 9 to 24°C (Fig. 4A, B). The specimens fed either T-ISO or the mixture T-ISO/T. *suecica*, spawned in total 59 and 58 times in the course of the 12 months, with an average of 1.13 and 1.12 times per week, respectively. The mean annual number of laid eggs was 681,000 and 687,000 per cockle for animals fed T-ISO and T-ISO/T. *suecica*, respectively (Table 2).

C. glaucum Sampled in the Wild

From January 1995 to October 1996, cockles were found in all stages of maturation, with individuals occurring in the undifferentiated to ripe stage at each time period (Fig. 5).

DISCUSSION

For many bivalves, the period for hatchery operators to obtain, condition, and spawn animals is short. This is because of different

factors, among which the most important are water temperature and feeding regime (Helm et al. 1973, Helm 1977, Rossi et al. 1994, Heasman et al. 1996, Wilson et al. 1996). Manzi and Castagna (1989) make exhaustive mention of difficulties met by bivalve mollusks hatchery operators when they want to prolong (anticipating and retarding) the natural spawning period. Alternative methods for extending spawning imply costly procedures for heating and cooling the water during the conditioning or importing the broodstock from lower or higher latitude sites. Beattie (1995) gives an economy view of broodstock management and shows that lower costs result when the parent bivalve mollusks are kept in captivity and conditioned, as compared to the continuous supply of wild caught broodstock. Our study shows that it is possible to get viable gametes from *C. glaucum*, and thereafter seeds, all year at a relatively wide range of temperatures (Fig. 4A, B).

As with most bivalve mollusks, the phase of maturation conditioning with selected algae feeding is obligatory (Millican and Helm 1994, Heasman et al. 1996). For *C. glaucum* with proper conditioning, as shown in experiment 3, it is possible to maintain viable broodstock for seed production all year. This study confirms that *D. tertiolecta* is of poor nutritional value for *C. glaucum*, as it is for other bivalve mollusks (Millican and Helm 1994). However, T-ISO is shown to be an adequate food source for the maturation conditioning phase as well as for prolonged spawning period of *C. glaucum*.

C. glaucum is widely distributed throughout Europe. Brock and Wolowicz (1994) mention that this species enters sexual maturation phase once a year in the Baltic Sea and twice a year in the north Mediterranean Sea. Zaouali (1975) states that the cockle has developed gonads all year in the lagoons of the coastal African Mediterranean Sea, with the exception of 1 month in autumn. Likewise, *C. glaucum* from Lesina Lagoon and border areas had sexually developed specimens all year (Fig. 5). Unfortunately, live cockles could not be obtained at each sampling interval in this study because of the high mortality of *C. glaucum* after prolonged anoxic period typical of eutrophic lagoon or the poor transparency of the water caused by wind generated resuspension of silt into the water column. From these findings, the reproductive potentiality of this filter feeder seem to be important in the trophic economy of the Lesina Lagoon in all seasons and makes this lagoon more

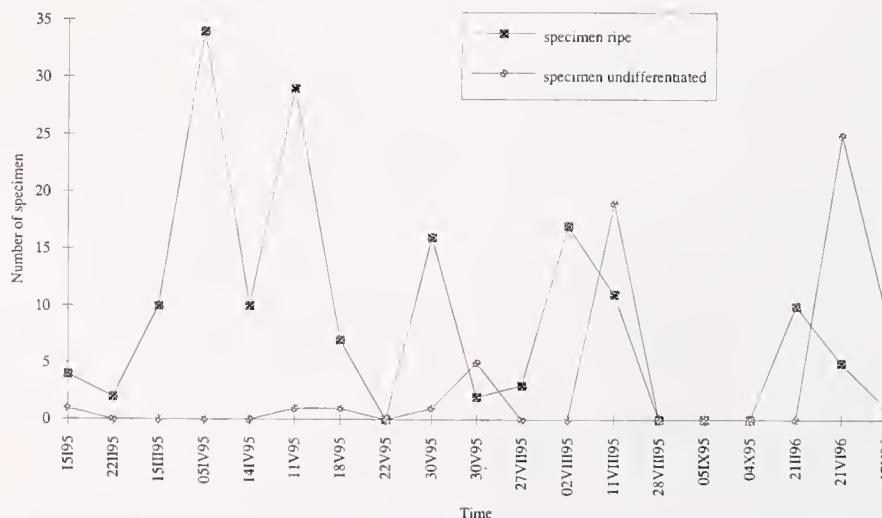


Figure 5. Maturation phase of gonads in *C. glaucum* sampled in the wild, during 2 years of survey. To facilitate the figure reading, fertile specimens from stage II to IV were reported as ripe.

similar to the southern Mediterranean wetland rather than to the northern one.

CONCLUSION

By conditioning cockles on T-ISO in the laboratory, cockles ripen and are able to produce viable seeds all year. In light of the fluctuating presence of live cockles and the abundance of empty shells in several places of the lagoon, it seems that, despite the high nutritional value of the suspended organic matter (organic particles and microphytoplankton) and the frequent finding of veliger stages in the plankton sampling (Cordisco 1996), the lagoon does not properly support the reproductive potentiality of this organism. Thus, this bivalve mollusk might play an important ecological role in eutrophic lagoons by converting the energy of organic particles into food for such cockle predators as sea bream (Barbaro et al. 1982). This cockle is found in the Lesina Lagoon in places with

different salinity (10 ppt and 50 ppt), in the ponds near Margherita di Savoia salterns (60 ppt), in the Bay of Cadiz (Spain) (50–60 ppt). It also inhabits such frontier as the Fortore River outlet, which undergoes frequent daily salinity changes (0 ppt to 36 ppt). Considering the temperature range tolerated by these cockles for thriving and spawning, in this study, it is intriguing to consider this bivalve mollusk as useful for restocking and production recovery of eutrophic lagoons and embayments with considerable loads of particulate organic matter.

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THE OCCURRENCE OF JUVENILES OF THE GRAPSID CRAB *CHASMAGNATHUS GRANULATA* IN SIPHON HOLES OF THE STOUT RAZOR CLAM *TAGELUS PLEBEIUS*

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ABSTRACT Previous samplings for megalopae and juvenile instars of the southwestern Atlantic burrowing crab *Chasmagnathus granulata* showed that they occur at high densities in adult crab burrows. Nevertheless, this study documents the occurrence of juveniles of this species in siphon holes of the stout razor clam *Tagelus plebeius* after a settlement event. Carapace width of the crabs collected ranged between 1.9 mm and 4.5 mm (2nd to 8th instar). A significantly higher proportion of crabs were found inhabiting inhalant instead of exhalant siphon holes. The siphon holes occupied by crabs showed a significantly larger diameter than those not occupied by them, and a significant positive correlation was found between siphon hole diameter and crab carapace width. A lateral chamber connected to the inhalant siphon gallery was observed in siphon holes inhabited by larger crabs. No correlation was found between the density of pairs of siphon holes and the proportion of pairs of siphon holes inhabited by crabs. However, the proportion of pairs of siphon holes occupied by crabs was higher in a site with low density of adult burrows in relation to adjacent sites with high density of adult burrows. We propose that the presence of clam siphon holes allows crab larvae to colonize poorly structured habitats where adult crab burrows are absent or at low densities, making possible the growth of areas inhabited by crabs.

KEY WORDS: Habitat structure, *Chasmagnathus granulata*, *Tagelus plebeius*, settlement, siphon holes

INTRODUCTION

In shallow marine environments, high densities of juvenile life stages or small-sized species of decapod crustaceans are commonly associated with habitats that show a complex tridimensional structure, such as cordgrass marshes (Zimmerman et al. 1983), seagrass meadows (Thomas et al. 1990), mangrove roots or canopies (Wilson 1989), tubiculous polychaete reefs (Gore et al. 1978), beds of mollusk shells (Gunderson et al. 1990), woody debris (Everett and Ruiz 1993), or cobble (Wahle and Steneck 1991). The habitat structuring elements serve as obstacles for predator activities and also act to minimize the injurious effects of the different disturbance sources and environmental extremes (Kneib 1984). Thus, mortality risk for these organisms is lower in structurally complex sites with respect to adjacent flat areas (e.g., Heck and Thoman 1981, Fernandez et al. 1993a). Although differential mortality after settlement may account for the higher density of decapods in structurally complex habitats (Johns and Mann 1987, Fernandez et al. 1993a), decapod larvae may actively select complex habitats as settlement sites (Botero and Atema 1982, Fernandez et al. 1993a, Fernandez et al. 1994). This preference may also be shown by juveniles (Johns and Mann 1987, Fernandez et al. 1993a).

The grapsid crab *Chasmagnathus granulata* Dana is one of the most abundant macroinvertebrates in saltmarsh and estuarine environments of the southwestern Atlantic (Boschi 1964). Its distribution ranges from Rio de Janeiro (23°S, Brazil) to the San Matías Gulf (41°S, Argentina; Boschi 1964). This gregarious species excavates and maintains semipermanent open burrows in the intertidal, from the soft bare sediment flats to areas vegetated by the cordgrass *Spartina densiflora* (Spivak et al. 1994, Iribarne et al. 1997), and behaves as deposit feeders in mud flats and as herbivorous-detritivorous in *S. densiflora* marshes (Iribarne et al. 1997). Because of their high density (up to 30 adults \cdot m⁻²; J. Gutiérrez pers. obs.) and the effect of their deposit feeding and burrowing activities on sediment composition (Botto and Iribarne 1997) and benthic community structure (Botto and Iribarne 1996), this species is likely to play a key role in determining the structure of SW

Atlantic marshes and estuarine soft-bottom communities. The stout razor clam *Tagelus plebeius* Solander is a deep burrowing bivalve species that constructs permanent burrows and siphon holes (Holland and Dean 1977). This species is distributed in estuarine environments of the western Atlantic coast from North Carolina (34°N, USA; Holland and Dean 1977) to the San Matías Gulf (41°S, Argentina; Iribarne and Botto 1998), attaining densities of 200 ind. \cdot m⁻² (Holland and Dean 1977, Iribarne et al. 1998) and coexisting with *C. granulata* in intertidal mud flat areas in most of their distribution range. Although there is no information, this coexistence pattern may produce several direct and indirect interactions.

Recently settled megalopae and juvenile *Chasmagnathus granulata* are found at high densities in association with adult crab burrows, and dense burrow beds seemed to be the major nursery habitat for this species (Spivak et al. 1994). However, during observations performed after a settlement event, we noticed the presence of juvenile *C. granulata* dwelling in siphon holes of *Tagelus plebeius*. Given that this type of shelter may be important, the purpose of this work is to describe the pattern of siphon hole use by juvenile *C. granulata*, focusing on: (1) size and molting instar of the crabs using the holes; (2) type of siphon hole occupied by crab (inhalant or exhalant); (3) differences between siphon holes occupied and not occupied by them; and (4) the percentage of holes occupied and density of crabs dwelling in siphon holes in crab beds with different densities of adult burrows.

MATERIALS AND METHODS

This study was carried out in the Mar Chiquita coastal lagoon (Argentina, 37°32' to 37°45' S, 57°19' to 57°26' W), a 46 km² body of brackish water (Fasano et al. 1982) affected by low amplitude (<1 m) tides (Lanfredi et al. 1987) and characterized by mud flats and a large surrounding cordgrass (*Spartina densiflora*) area (Olivier et al. 1972, Iribarne et al. 1997). In soft sediment flat areas of the lagoon, the spatial coexistence between *Chasmagnathus granulata* and *Tagelus plebeius* is a common phenomenon (e.g., Olivier et al. 1972). Samplings were conducted during the

late summer of 1998 in two areas (located approximately 10 m apart) at the same intertidal level (0.5 m above mean lower low water [MLLW]). Both sites are characterized by sandy-silty sediments and the presence of sediment depressions (often associated with *C. granulata* burrows) whose sediment shows a higher organic matter content with respect to those of the surrounding mud flats. However, they differ in the density of *C. granulata* burrows (high burrow density site [HBD site]: $x = 6.98$ burrows $\cdot m^{-2}$, $SD = 5.41$; low burrow density site [LBD site]: $x = 0.58$ burrows $\cdot m^{-2}$, $SD = 1.72$) and the percentage of area covered by sediment depressions (HBD site: $x = 51.2\%$, $SD = 25.4$; LBD site: $x = 3.2\%$, $SD = 3.01$; Gutiérrez and Iribarne unpubl. data).

A random sampling using 4 m² square sampling units (SU) was conducted both at the HBD ($n = 8$) and LBD site ($n = 10$). The total number of pairs of clam siphon holes at each SU was counted. At the LBD site, 10 pairs of clam siphon holes were randomly taken from each SU. Inhalant and exhalant holes were determined by the presence of pseudofeces, and their diameters were measured to the nearest 0.01 mm. Later, the 10 pairs of measured siphon holes were carefully excavated. All crabs obtained were carried to the laboratory to be determined to the species level, and their carapace widths (CW) measured to the nearest 0.01 mm. The presence or absence of lateral burrow chambers was recorded. The remaining pairs of siphon holes of each SU of the LBD site and all the pairs of siphon holes of the HBD site were also excavated but only to determine presence or absence of crabs. The null hypothesis of no difference in the number of pairs of siphon holes between the HBD and the LBD site was contrasted with the Mann-Whitney test (Conover 1980). A binomial test (Conover 1980) was used to test for differences in the proportion of crabs occupying siphon holes corresponding to the inhalant or exhalant siphon. A Wilcoxon test (Conover 1980) was used to evaluate for differences in the diameter between the hole occupied by the crab and the complementary of the pair, and a Mann-Whitney test (Conover 1980) was employed to test for differences in the relation: diameter of the major siphon hole/diameter of the minor siphon hole between crab inhabited and vacant pairs of siphon holes. A correlation analysis (Zar 1984) was performed to evaluate the existence of an association between crab size (CW) and hole diameter. The null hypothesis of no difference in the size (CW) between crabs occupying holes with and without lateral chambers was contrasted using Student's *t*-test (Zar 1984). The possibility of association between the number of siphon holes in a SU and the number of crabs per pair of siphon holes was evaluated using a correlation analysis (Zar 1984). A Mann-Whitney test (Conover 1980) was applied to test for differences in the relation: number of siphon holes occupied by crabs/total number of siphon holes and in the density of crabs occupying holes between the HBD and the LBD sites. Shapiro-Wilk tests (Zar 1984) were used to test for normality of the data, and Levene's test (Underwood 1997) was applied to test for homocedasticity of the data. In those cases where sample sizes were unequal, the calculation of the test statistics were carried out using the corrections suggested by Conover (1980) and Zar (1984).

RESULTS

The density of pairs of siphon holes of *Tagelus plebeius* differed significantly between sites (HBD: $x = 43.56$ pairs $\cdot m^{-2}$, $SD = 5.41$; LBD: $x = 12.86$ pairs $\cdot m^{-2}$, $SD = 10.06$; Mann-Whitney test: $Z = -6.42$, $p < .01$). Carapace width of the crabs collected ranged between 1.9 and 4.5 mm (2nd to 8th instar; ac-

cording to Rieger and Nakagawa 1995). A significantly higher proportion of crabs (86.67%) was encountered dwelling in the inhalant siphon holes (Binomial test: $t_1 = 26$, $t_2 = 4$, $n = 30$, $p < .001$) than in the exhalant hole. The siphon holes inhabited by crabs showed significantly larger diameter than their complement (crab: $x = 5.81$ mm, $SD = 1.62$; no crab: $x = 4.46$ mm, $SD = 0.8$; Wilcoxon test: $Z = 4.31$, $p < .001$; Fig. 1A), and the ratio between the diameters of the major hole and the minor hole was significantly higher in pairs of siphon holes occupied by crabs (crab: $x = 1.31$, $SD = 0.27$; no crab: $x = 1.06$, $SD = 0.04$; Mann-Whitney test: $Z = -6.85$, $p < .001$; Fig. 1B). A significant positive correlation was encountered between crab carapace width and diameter of the siphon hole occupied by crabs ($r^2 = 0.80$, $df = 1$, 28 , $p < .001$, Fig. 2). 36.67% of the crabs construct lateral chambers associated to the siphon hole at depths ranging between 4.5 and 8 cm, and all the chambers were found in association with the inhalant tube. The crabs found in holes with chamber showed significantly larger carapace width with respect to those dwelling in holes without (chamber: $x = 3.86$ mm, $SD = 0.51$; no chamber: $x = 2.76$ mm, $SD = 0.5$; *t*-test: -5.78 , $df = 28$, $p < .001$; Fig. 3). No significant relationship was found between the number of pairs of siphon holes in the sampling units and the number of pairs inhabited by crabs ($r^2 = 0.07$, $df = 1$, 19 , $p > .05$). The percentage of pairs of siphon holes occupied by crabs was significantly higher at the LBD site (LBD: $x = 31.24\%$, $SD = 5.38$; HBD: $x = 8.52\%$, $SD = 2.34$; Mann-Whitney test: $Z = -3.55$, $p < .001$; Fig. 4). However, the density of crabs occupying holes did not differ between

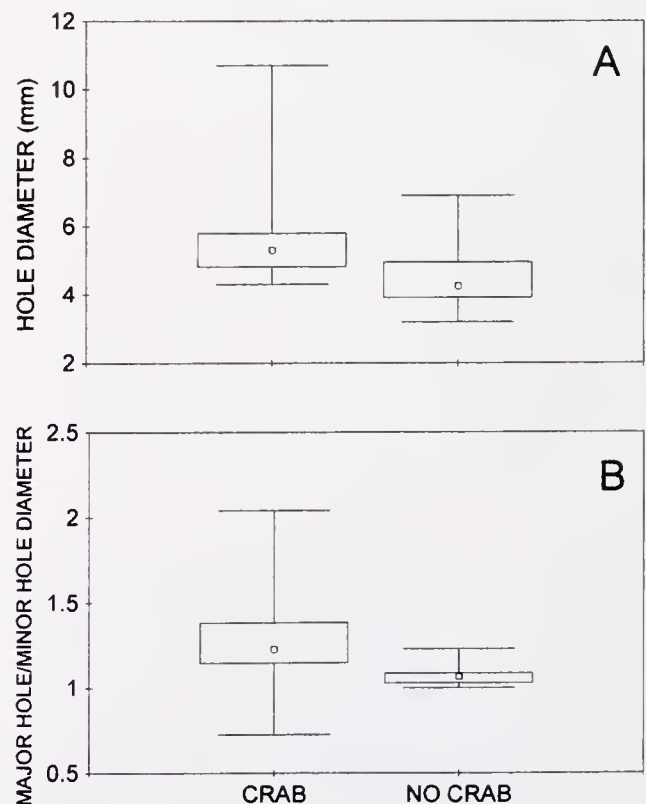


Figure 1. Median quantile box plots showing: (A) the diameter of the siphon holes occupied by juvenile *Chasmagnathus granulata* and the complementaries of each pair; and (B) the ratio between the diameter of the major and minor hole for pairs of siphon holes with and without crab.

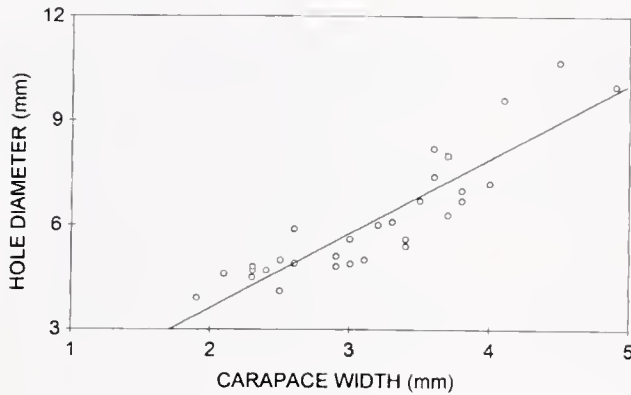


Figure 2. Carapace width of juvenile *Chasmagnathus granulata* against diameter of the siphon hole occupied by them.

sites (LBD: $x = 3.87 \text{ crabs} \cdot \text{m}^{-2}$, $SD = 0.83$; HBD: $x = 3.4 \text{ crabs} \cdot \text{m}^{-2}$, $SD = 1.51$; t -test: $t = 0.79$, $df = 16$, $p > .05$).

DISCUSSION

Larval decapod settlement is primarily an active process in which larvae choose settlement sites based on sediment characteristics or chemical cues (Castro 1978, Botero and Atema 1982). Flume experiments demonstrated that megalopae of *Chasmagnathus granulata* actively swim in current conditions similar to those encountered in the field, and it was also proposed that they are able to select settlement sites (Valero 1998). In addition, the higher densities of recently settled megalopae and juveniles occur in association with adult crab burrows (Spivak et al. 1994), and metamorphosis of *C. granulata* megalopae occurs earlier in the presence of chemical cues of adult conspecifics than with sea water alone (Gebauer et al. 1998). All these data suggest that megalopae of this species settle in response to adult-released chemical cues. In this context, the occurrence of juvenile *C. granulata* dwelling in siphon holes of stout razor clams cannot be explained by means of selective settlement of megalopae. An alternative hypothesis may be stated on the basis of competition between cohorts. Settlement of *C. granulata* megalopae occurs in the lagoon between December and June, with peaks in intensity (Spivak 1994). Thus, there is a potential for interaction between cohorts. Early cohorts of the Dungeness crab *Cancer magister* reduces the abundance of subsequent cohorts in intertidal shell habi-

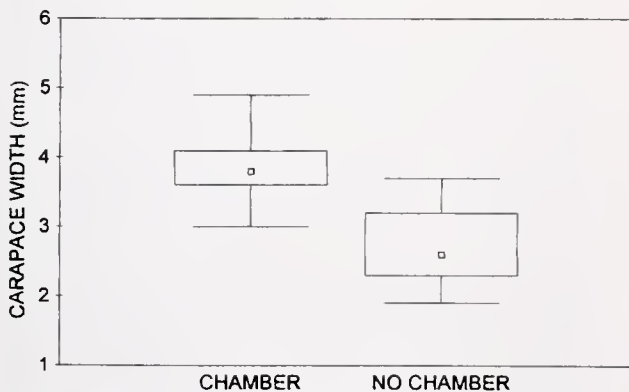


Figure 3. Median quantile box plots showing carapace width of juvenile *Chasmagnathus granulata* occurring at siphon holes with and without lateral chambers.

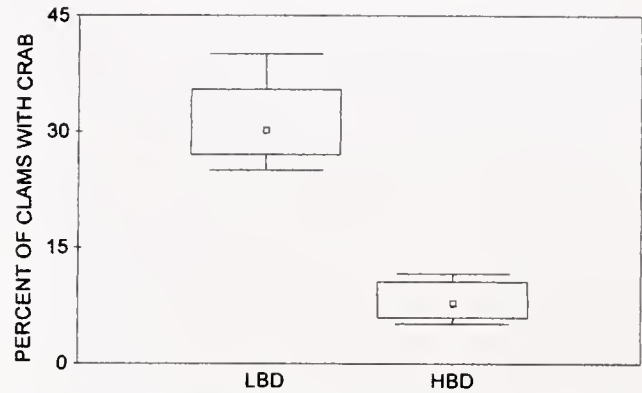


Figure 4. Median quantile box plots showing the proportion of pairs of siphon holes occupied by juvenile *Chasmagnathus granulata* at the site of low (LBD) and high density of adult burrows.

tats, and smaller juveniles migrate to the open flats in response to high density of conspecifics (Fernandez et al. 1993b). Early juvenile instars of *C. granulata* are subject of cannibalism by larger juveniles (Luppi et al. 1995), and it may be possible that a density-dependent migration from adult burrows to siphon holes has been carried out by crabs settling in late summer.

Nevertheless, the possibility of direct settlement of *C. granulata* megalopae in siphon holes cannot be discarded. Muddy sand substrata (such as those occurring at both study sites) also promotes an earlier metamorphosis of *C. granulata* megalopae when compared with coarser sediment types. Despite the fact that a combination of muddy substrata and adult chemical cues determines the shortest time to metamorphic molt, muddy sand substrata alone have an important effect (Gebauer et al. 1998). If we assume that muddy sand substrata alone may also induce settlement of *C. granulata* megalopae, settlement in sites other than adult burrows is plausible. In addition, Gebauer et al. (1998) found that artificial substrata of the same grain size do not have the same effect on metamorphic molt, suggesting that characteristics other than grain size are relevant for the induction of metamorphosis (see Pawlik 1992). As previously mentioned, sediment from adult burrows and sediment depressions in association with adult burrows are organically richer than those of the surrounding flat. Taking into account that this species behaves as a deposit feeder in unvegetated sediment flats, it is likely that high levels of organic matter may function as a cue for the settlement of megalopae of this species. Although no data are available about the small-scale distribution of organic matter around bivalve siphon holes, increased levels may occur as a result of the enhancement of local particle deposition caused by flow convergence toward the inhalant siphon (see Ertman and Jumars 1988). Moreover, larvae may respond to physical factors (see Pawlik 1992). Negative bottom roughness elements (such as burrows and siphon holes) behave as hydrodynamically quiet microhabitats (DePatra and Levin 1989). Weak current conditions are commonly associated with habitats that have a complex tridimensional structure (e.g., oyster beds, Wright et al. 1990), which may provide refuge for juvenile decapods. Dungeness crab *C. magister* megalopae are able to select positively for weak current conditions (e.g., Fernandez et al. 1994). In addition, metamorphosis of the blue crab *Callinectes sapidus* megalopae is accelerated by textural cues caused by the presence of eelgrass (Forward et al. 1994). Burrows or siphon holes may produce similar effects on *C. granulata* megalopae.

Siphon holes occupied by juvenile *Chasmagnathus granulata* appeared modified with respect to the complementarity of the pair. These crabs enlarge the diameter of the siphon holes occupied by them and larger crabs also construct lateral chambers. This may occur as a result to the provision of space for both the crab and the extended siphon. The thalassinidean shrimp *Jaxea nocturna* inhabit burrows of the echiurid *Maxmuelleria lankesteri*, modifying them by the excavation of semicircular side branches (Nickell et al. 1994). In addition, it was proposed that *J. nocturna* probably benefit by the irrigation activities of the echiuran, which supply both oxygen and food (Nickell et al. 1994). Pinnotherid crabs *Pinnixa schmitti* and *Scleroplax granulata* behave as symbionts in burrows of the suspension feeding burrowing shrimp *Upogebia pugettensis* of the Northwestern Atlantic (Kozloff 1987). Thus, the higher proportion of juvenile *C. granulata* occurring in siphon holes corresponding to the inhalent siphon may be the result of selectively favoring a site with a high inflow of food particles. However, we do not know to what extent clam attributes are affected (e.g., decreased filtration and oxygen consumption rates in mussels with pea crabs in the mantle cavity; Biernbaum and Shumway 1988), to determine if the relationship is commensalistic or parasitic.

It is also interesting to notice that the percentage of holes occupied by juvenile *Chasmagnathus granulata* was higher in the site with a low density of adult burrows than in the site with a high density of adult burrows. This pattern indicates that where habitat structure is poor, small scale sediment features may be important as settlement sites and/or refuges for this species. We also believe that the strategy of settling into holes of bivalves or other burrowing species may be a fairly common phenomenon. For example, new settlers of the Dungeness crab *Cancer magister* have been found in holes of burrowing shrimps in the Grays Harbor estuary (Washington, USA; O. Iribarne, pers. obs.) but their importance has never been quantified.

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POPULATION BIOLOGY OF *XIPHOPENAEUS KROYERI* (HELLER 1862) (DECAPODA: PENAEIDAE) FROM UBATUBA BAY, SÃO PAULO BRAZIL

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ABSTRACT The population structure and abundance of *Xiphopenaeus kroyeri* (Heller, 1862) were analyzed during monthly samples from October 1992 to September 1993 in Ubatuba Bay (23°26'S and 45°02'W), Brazil. Sampling was carried out at two parallel transects: one of them located in its midregion and the other at the mouth of the bay. After trawling, shrimps were separated from other benthic organisms, sexed, and counted. Their total length was also measured, and the degree of gonadal development was assessed. *Xiphopenaeus kroyeri*, the most common penaeid species in the bay, was recorded in all samples, but its abundance decreased from November to March. Size ranged from 14.3 to 118.3 mm in males and from 12.7 to 133.6 mm in females, suggesting a slight sexual dimorphism related to body size. Males prevailed during most of the year; whereas, females predominated during summer and midwinter. Based on the percentage of mature females during this study, two main reproductive periods were identified, occurring in spring and autumn. Despite some breeding activity throughout the year, such a trend indicates that the population follows a tropical/subtropical reproductive pattern.

KEY WORDS: Population biology, Penaeidae, *Xiphopenaeus kroyeri*, reproduction, sex-ratio

INTRODUCTION

Xiphopenaeus kroyeri is the most intensively exploited shrimp species in São Paulo State. According to Pires (1992), *X. kroyeri* and the swimming crab *Portunus spinicarpus* (Stimpson 1871) are the most abundant species of the benthic megafauna in the continental shelf off the study area. This species represents the second most important fishery resource along the coast of São Paulo State (Rodrigues et al. 1993), and its trophic relationships may be essential in maintaining the stability of benthic communities (Pires 1992). Despite being an extremely abundant species along the Brazilian coast, information on the biology, ecology, and behavior of *X. kroyeri* is scarce (Vieira 1947, Mota-Alves and Rodrigues 1977, Cortés and Criales 1990, Cortés 1991, Rodrigues et al. 1993 and Branco et al. 1994).

Because of the complexity of their life cycle, studies on penaeid shrimp populations (e.g., migration and reproduction) are needed to improve fishery management. Boschi (1969) pointed out its importance when he studied *Artemesia longinaris* Bate 1888 in Mar del Plata, noting a continuous change of its age composition structure.

The study of penaeid reproductive cycles is also important, and usually is achieved by means of recording the degree of gonadal development in sampled specimens. In this procedure, a number of development levels are established and described, usually ranging from three to five (i.e., immature, in maturation, almost mature, mature, and spawned). Relevant contributions concerning the reproductive biology of different penaeid shrimp species are Vieira (1947), Olguín-Palacios (1967), Perez-Farfante (1969), Mota-Alves and Rodrigues (1977), Motta-Amado (1978), and El Hady et al. (1990). This study examines the abundance and the population biology of *X. kroyeri* in Ubatuba Bay, Ubatuba, São Paulo, Brazil, with emphasis on its population structure and reproductive period.

MATERIALS AND METHODS

Monthly trawlings were carried out from October 1992 to September 1993 in Ubatuba Bay. Samples were taken along two

transects: transect A, located at the shallow midregion of the bay, and transect B located at the deeper bay mouth (Fig. 1). Trawlings were accomplished with an otter trawl net (10-mm mesh cod end) and were conducted at constant speed for 1 h, covering a 7,400 m² area. The shallow region of the bay is strongly affected by coastal environmental conditions, receiving freshwater drainage from four rivers. Otherwise, the deep stratum is to subject a greater oceanic influence.

Some physical factors were monitored at each transect. Bottom water temperature was obtained with a Nansen bottle provided with a thermometer ($\pm 1.0^{\circ}\text{C}$). Depth was obtained by means of a marked rope attached to the Nansen bottle, and a VanVeen grab sampler was used to obtain sediment samples. Sieving analysis according to Wentworth grades were carried out for grain size classification. Sediments were sorted using the phi-scale (ϕ) in 1.0- ϕ intervals between -1.0ϕ and 4.0ϕ , including the fractions under 4.0ϕ (Hakanson and Jansson 1983). Sediment organic contents were obtained using the loss on ignition method (Hakanson and Jansson 1983).

The shrimps were separated, sexed, and measured (total length, TL) with a vernier caliper to the nearest 0.1 mm. A Student's *t*-test was used to detect size differences between sexes. Box plots were performed for males and females in each month to analyze the population structure through the study period. Monthly sex ratios were also obtained.

Median size differences between transects were tested in each month using a Mann-Whitney U-test and among months in each transect using a Kruskal-Wallis test (Siegel, 1956).

In each trawl, subsamples from 200 to 400 individuals were separated for examination of gonads. In females, four development stages were considered according to Motta-Amado (1978): I-immature, II-developing, III-mature, and IV-spent. In males, the presence (or absence) of spermatophores in the terminal ampoule was recorded.

Monthly sex ratios and proportions of mature females were statistically compared by means of a Goodman's test (1964, 1965).

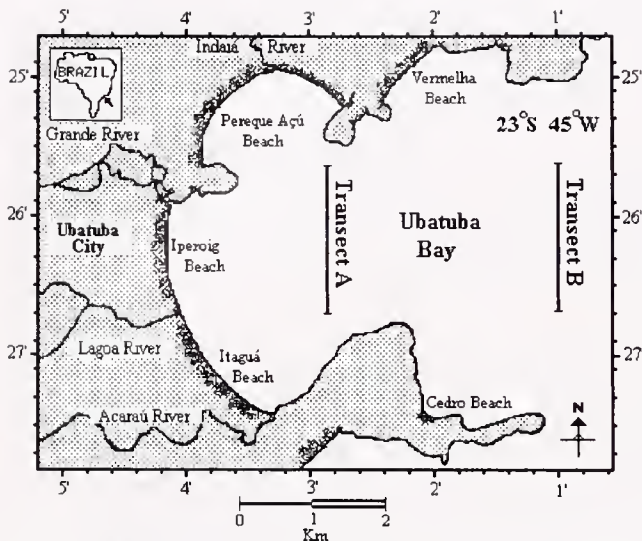


Figure 1. Map of Ubatuba Bay showing the position of sampling transects.

This analysis is based on the binomial proportion comparison for contrasts between and within multinomial populations. These results were analyzed at the 5% significance level.

To estimate the mean size of first maturation in each sex, the Galton's Ogive, $Fr = 1 - e^{-a \cdot TL^b}$ (Fonteles-Filho 1989), was adjusted to fit the total length (TL, independent variable) versus relative frequency of mature individuals (Fr, dependent variable) scatterplots. Mature individuals were defined as male with full terminal ampoule and females with gonads in stage II, III, or IV.

RESULTS

Water temperature averaged $23.16 \pm 2.97^\circ\text{C}$ (ranging from 20 to 28°C), with highest values recorded from February to April. Mean depth at transect A is 7.6 ± 1.3 m. Along this transect sediments are very poorly sorted ($\sigma_1 = +2.025$), mainly consisting of fine sand ($Mz = 2.73 \phi$) with a high percentage of organic contents ($11.66 \pm 2.14\%$). Average depth at transect B is 14.6 ± 0.74 m. Sediments are moderately sorted ($\sigma = +0.525$), composed by very fine sand ($Mz = 3.40 \phi$) and low organic contents ($2.97 \pm 0.61\%$).

Xiphopenaeus kroyeri was very abundant in the area (5,027 individuals in transect A and 5,282 in transect B) during all sampling periods, except from December to February in transect B (Fig. 2). This fact indicates a seasonal abundance variation.

Females ranged from 12.7 to 133.6 mm (74.52 ± 17.47) and

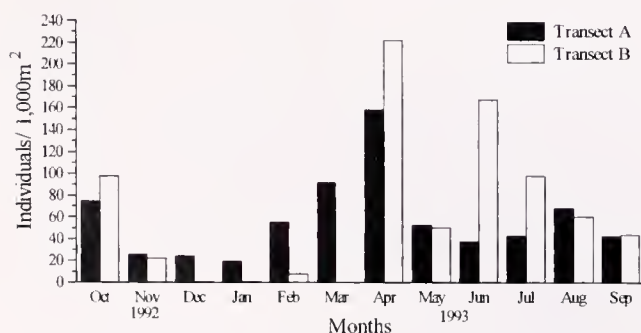


Figure 2. Monthly abundance of *X. kroyeri* in both transects.

males from 14.3 to 118.3 mm (71.82 ± 14.01), indicating a sexual dimorphism, in which females attain a larger size (Student's *t*-test, $t = 8.825$, $p < .0001$).

Mann-Whitney comparative analyses of size frequency distributions in transects A and B revealed significant size differences in Oct. 1992, Apr., May, June, and Aug. 1993 (Table 1, Figs. 3 and 4), showing that the population is not equally distributed in these areas. During Oct. 1992, April, and June 1993, larger shrimps were sampled in transect A, and during May and Aug. 1993, larger specimens were captured in transect B.

Differences in shrimp median size were repeatedly verified among sampled months (Kruskal-Wallis, $p < .001$) (Table 2, Figs. 3 and 4). However, they were too complex to reveal recruitment pattern.

Males were generally slightly predominant, but the sex ratio varied throughout the year. In November 1992, January and July 1993 (Fig. 5), an increase (Goodman's test, $p < .05$) of the relative number of females was observed, when sex ratios attained 1:1.78; 1:1.28, and 1:1.2, respectively.

Xiphopenaeus kroyeri breeds all year, but higher reproductive activity was verified in some periods. Based upon the data obtained from gonadal analysis in females, it can be concluded that higher reproductive activity occurred in November 1992, and March, August, and September 1993 (Fig. 6). In the case of males, higher proportions of individuals with full terminal ampoule were recorded in two main periods (November 1992 and May 1993) (Fig. 7). Males achieve sexual maturity (68.02 mm) at a smaller size than females (83.19 mm) (Fig. 8).

DISCUSSION

The abundance of *X. kroyeri* showed a marked seasonal variation. During the summer period (December to March) this species' abundance is lower, mainly in the deeper portion of the bay. Signoret (1974) observed that *X. kroyeri* follows a similar pattern in the Terminos Lagoon (México), with low abundances from summer to fall.

The low abundance of *X. kroyeri* during summer may be related to the intrusion of a cold current. Castro-Filho et al. (1987) indicated the presence of three oceanic currents in the Ubatuba region, the coastal water (CW) ($T > 20^\circ\text{C}$), the South Atlantic central water (SACW) ($T < 18^\circ\text{C}$), and the tropical water (TW) ($T > 20^\circ\text{C}$). Pires (1992), who studied the benthic megafauna communities in the continental shelf of Ubatuba region, observed by means of a cluster analysis that there is a close association between some species abundance and specific environmental conditions. This is the case of positive correlation between *X. kroyeri* and the prevalence of CW during winter.

Despite different sediment features found in each transect, the incoming SACW during summer could be the most important physical factor influencing the distribution of this species. The physical action of the current itself together with low temperature conditions would restrain the population distribution of *X. kroyeri* within the study area.

Statistical differences in shrimp median size among sampled months do not support a growth model through time, which could have explained the growth pattern in this population. The great fishery effort in Ubatuba Bay probably affects the species, as observed by Somers et al. (1987) in *P. esculentus* Haswell in the Torres Strait (Australia), who suggested a continuous recruitment and/or the existence of a size-dependent source of mortality. In the present study, the comparison of shrimp size in transects A and B,

TABLE 1.

Mann-Whitney analysis in *X. kroyeri* size comparisons in each month between transects in Ubatuba Bay.

Month	Transect A		Transect B		U
	Number of individuals	Ranked sums	Number of individuals	Ranked sums	
October 1992	552	735.56	719	559.57	8.47*
November 1992	193	180.08	163	176.63	0.31
December 1992	184	95.00	7	122.28	1.28
January 1993	142	73.79	4	63.25	0.50
February 1993	409	229.59	52	242.08	0.63
March 1993	673	—	0	—	—
April 1993	1169	1568.07	1640	1288.34	8.996*
May 1993	390	351.24	370	411.34	3.77*
June 1993	275	816.52	1235	741.91	2.56*
July 1993	281	509.77	695	479.90	1.50
August 1993	466	381.11	401	495.46	6.70*
September 1993	293	296.16	296	293.85	0.16

* Statistical significant differences at $\alpha = 0.01$.

reveals remarkable differences in the population distribution, which are likely to reflect recruitment and migration processes.

The sex ratio variations observed in this study are supported by other results obtained for the same species. According to Signoret (1974), the sexual distribution through the year in *X. kroyeri* is not homogeneous, with males and females often strongly segregated.

According to Wenner (1972), mentioning the Fisher theory, the 1:1 proportion is favored by natural selection. Wenner (1972)

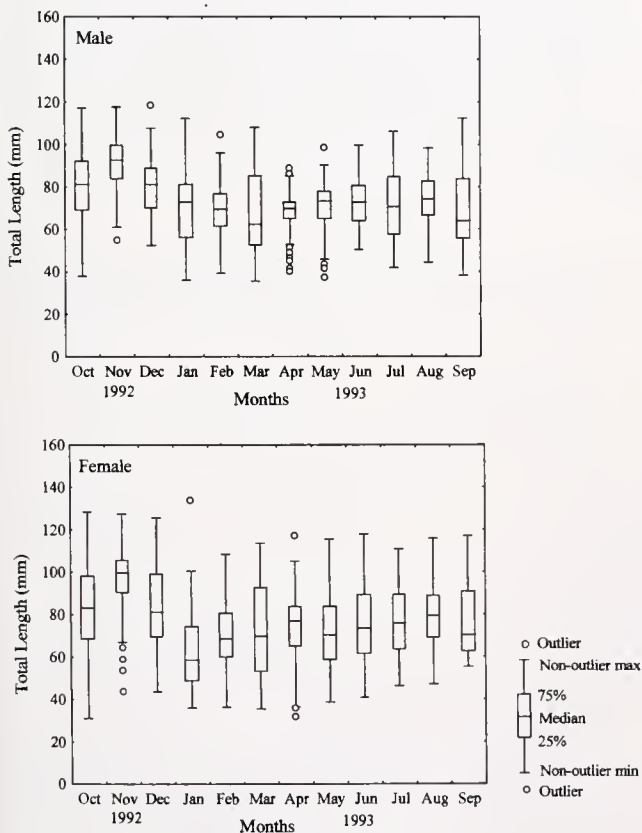


Figure 3. Series of box plot graphics for monthly size of males (upper) and females (below) obtained in transect A.

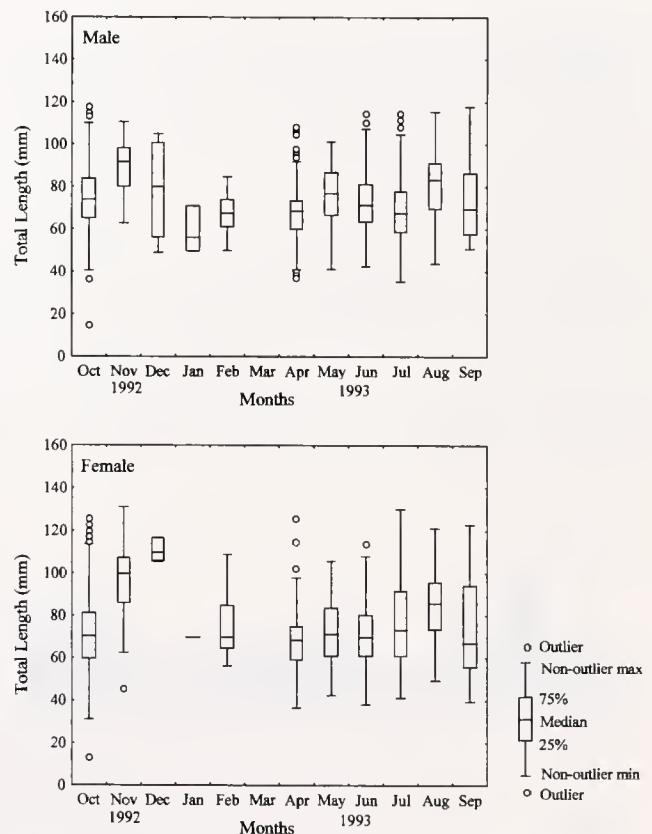


Figure 4. Series of box plot graphics for monthly size of males (upper) and females (below) obtained in transect B.

TABLE 2.
Results of Kruskal-Wallis analysis.

Transect	n	H	df	Probability
A	5027	576.25	11	$p < .001$
B	5282	640.68	10	$p < .001$

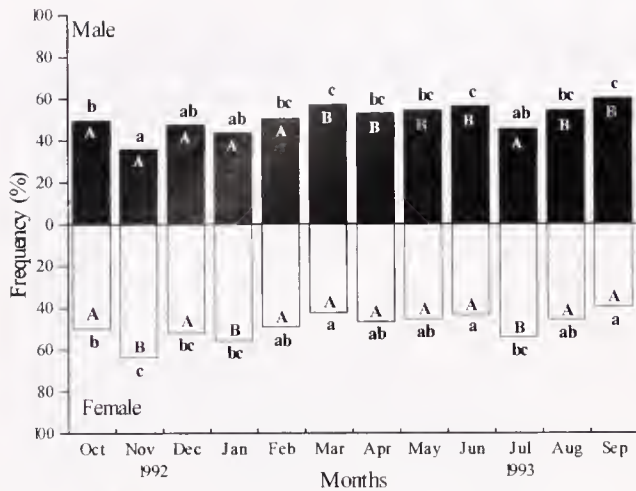


Figure 5. Monthly sex ratio for *X. kroyeri*. Capital letters inside the bars indicate comparisons within month, and lower case letters outside the bars indicate comparisons among months. The same letters indicate no statistical differences.

pointed out that sex-dependent mortality, activity, migration, habitat utilization and also the effect of restricted food resources are important factors explaining departures from the Mendelian proportion. Wenner also stated that the 1:1 ratio is an exception rather than the rule in crustacean populations. He concluded that sex ratio can be a function of size for a given species.

The temporal sex ratio variation can be related to a seasonal reproductive pattern in *X. kroyeri*. The proportion of males was higher during most of the year, but females were more abundant in November (spring), when it was recorded a 1:1.78 sex ratio. This peak coincides with major reproductive activity. Contrarily, Cortés (1991) observed that during spawning, males outnumbered females in a Colombian Caribbean population. This fact supports the hypothesis of sex-dependent pattern of migration, because Cortés collected the shrimps near the coast at depths ranging from 1.5 to 3 m.

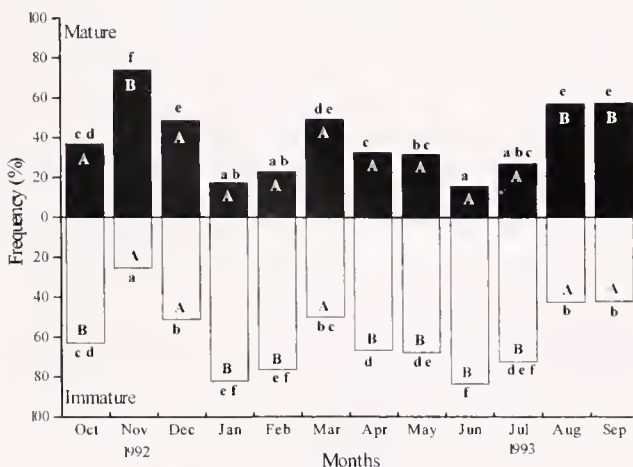


Figure 6. Bar graph showing gonadal maturity in females. Capital letters inside the bars indicate comparisons within month, and the lower case letters outside the bars indicate comparisons among months. The same letters indicate no statistical differences. Data of transects A and B are grouped. Females in gonad stage I were considered immature, and females in stages II, III, and IV were considered mature.

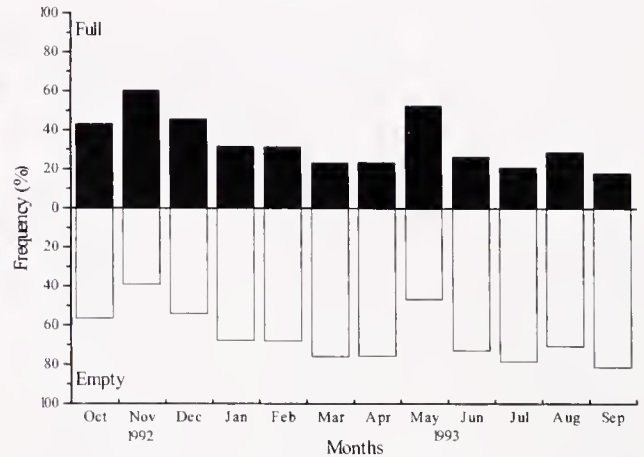


Figure 7. Terminal ampoule status in males.

Analyzing both transects, we can assume that *X. kroyeri* exhibits a tropical/subtropical reproductive pattern (Dall et al. 1990), in which there is a main reproductive period in the spring and a secondary one in the fall. The present results are similar to those observed by Mota-Alves and Rodrigues (1977), Motta-Amado (1978), and Cortés (1991). It can be assumed that the presence of juvenile individuals from February to May is attributable to spawning events during spring.

The onset of sexual maturity can vary between populations. The size estimates in this study (68.02 mm in males and 83.19 mm in females) are larger than those observed by Rodrigues et al. (1993) (62 mm for males and 71 mm for females) in other localities within São Paulo State. The determination of this parameter can be important for assessment of the reproductive stock in natural populations (Fonteles-Filho 1989) and for guidance of future governmental fishery control. Establishing a minimum catch size and defining the period of lower relative abundance of juvenile shrimps will help establish rational management of this species' exploitation.

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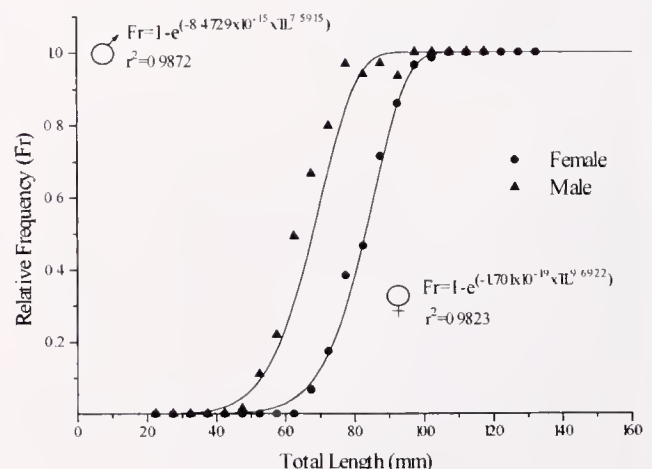


Figure 8. *X. kroyeri*. Frequency of morphologically mature males and females as a function of total length.

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PRODUCTION AND RESOURCE ALLOCATION IN THE PERIWINKLE, *LITTORINA LITTOREA* (LINNAEUS 1758), ON PENDLETON ISLAND, NEW BRUNSWICK

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ABSTRACT An intertidal population of *Littorina littorea* L. from a rocky shore on Pendleton Island, New Brunswick, Canada was analyzed to determine its demographic structure and the energy allocated to gamete production, somatic growth, and the synthesis of organic shell matrix. Total production, calculated as the sum of somatic growth (Pg), shell growth (Ps), and the production of gametes (Pr), was 151 kJ/m²/y⁻¹. The percentage of production allocated to Pg, Ps, and Pr was 60.0, 5.9, and 36.1%, respectively. Younger cohorts (0 and 1) were responsible for the bulk of shell (41.5 and 20.5%, respectively) and somatic production (58.5 and 79.5%, respectively), but for 0% of the reproductive output. As compared to other populations of *L. littorea*, the proportion of total production allocated to gamete production by the population on Pendleton Island was higher.

KEY WORDS: *Littorina littorea*, production, resource allocation, reproduction

INTRODUCTION

Estimates of production are useful in assessing contributions of marine species to energy flow through the ecosystem (Rodhouse 1979, Griffiths 1981a, Griffiths 1981b, Vahl 1981) in addition to determining the suitability of different habitats to producers (Bayne and Worrall 1980). Among mollusks, bivalves have received the most attention (*Cerastoderma edule*, Ivell 1981, *Placopecten magellanicus*, MacDonald and Thompson 1986, *Mytilus edulis*, Bayne and Worrall 1980, Thompson 1979, and Gardner and Thomas 1987a) largely because of their commercial and ecological importance.

The common periwinkle, *Littorina littorea* (L.), is one of the most widely studied marine gastropods. Extensive literature exists regarding its biology (Gegenbaur 1852, Caullery and Pelsener 1910, Linke 1933, Fretter and Graham 1962), breeding cycle (Tattersall 1920, Elmhirst 1923, Moore 1937, Williams 1964, Fish 1972, Chase and Thomas 1995a) and growth (Hayes 1927, Moore 1937, Ekaratne and Crisp 1982, Gardner and Thomas 1987b). However, there is a paucity of data on its production and allocation of resources. Grahame (1973) examined the importance of reproduction as a pathway of energy flow when he calculated both the production of somatic tissue and gamete production of *L. littorea* in North Wales. Hughes and Roberts (1980) compared the reproductive effort of *L. littorea* to that of *L. neritoides*, *L. nigrolineata* and *L. rudis* in North Wales. In Canada, the only study that has calculated secondary production of *L. littorea* was that of Gardner and Thomas (1987b) on a population at Welch's Cove, Bay of Fundy. In their study, however, Gardner and Thomas (1987b) considered only the partitioning of energy between somatic and shell growth; reproductive output was not considered. No study to date has looked at all components of production (somatic growth, shell growth, and the production of gametes) and the allocation of resources to those components. *L. littorea* is the most dominant mollusk within the midlittoral zone of rocky shores on Pendleton Island and throughout most of the Bay of Fundy (Gardner and Thomas 1987b). Knowledge of the production and allocation of resources in populations of *L. littorea* will provide information critical to the understanding its life history, and importance to the rocky shore community. The objective of this paper is twofold. The first is to calculate secondary production as the sum of all

components (somatic, shell, and gamete) to determine the proportion of total production allocated to gamete production. The second is to determine the age-specific pattern of energy partitioning between growth (somatic and shell) and reproduction.

MATERIALS AND METHODS

Study Site

Pendleton Island is located in New Brunswick, Canada at 45°02'N and 67°56'W within the Deer Island Archipelago (Fig. 1). The climate and oceanographic setting have been summarized by Thomas et al. (1990). The collection sites were on an approximately 100 m long section of shore in Pendleton Passage, a sheltered channel with high tidal currents ranging up to 0.47 and 0.60 m/s⁻¹ for the ebb and flood of spring tides, respectively (Thomas et al. 1990). The upper shore is predominately sedimentary, with scattered rocks and outcrops, and *L. littorea* are abundant throughout this area.

Demographics

Monthly samples of *L. littorea* were collected between May and September, 1988 and May and October, 1989. Samples comprised approximately 1,000 individuals, collected randomly from all tidal levels along three transects. The spire height was measured using computer-assisted vernier calipers. Gardner (1986) has shown that age of *L. littorea* cannot be ascertained accurately from growth ring analysis. Therefore, length frequency histograms were plotted, and the cohort placement of Cassie (1954) was used to analyze the demographic structure of the population. Although it is accepted that cohort identification by such a means may be subjective, we feel that regular sampling of the population and the incorporation of a settlement study (Chase and Thomas 1995a) more than compensated for the subjectivity of this method.

Production

Secondary production was calculated using the following relationship,

$$P = Pg + Pr + Ps \quad (1)$$

where; P = total production; Pg = energy incorporated into so-

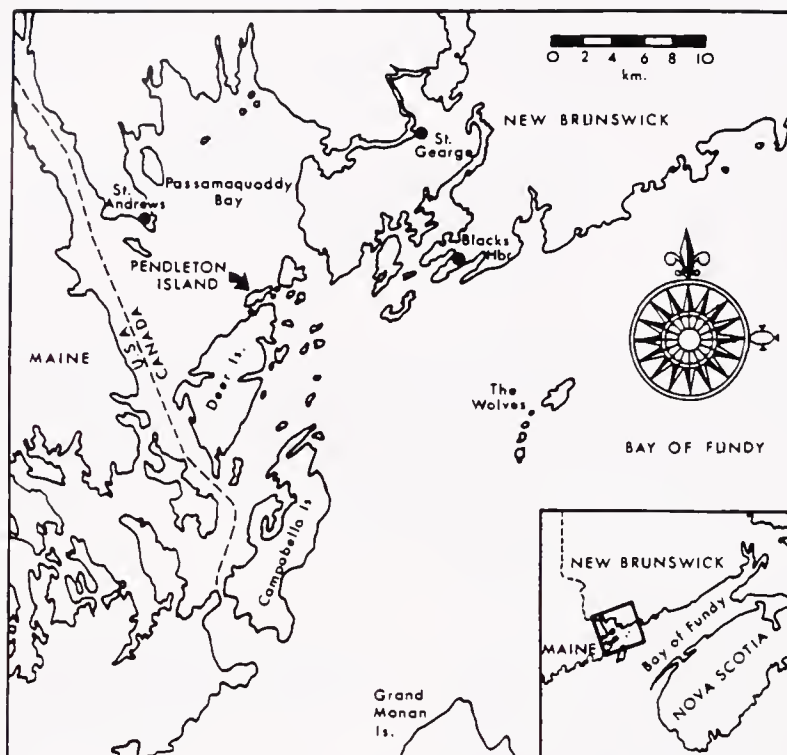


Figure 1. Location of Pendleton Island in the Deer Island Archipelago, New Brunswick, Canada.

matic growth; P_s = energy incorporated into the shell matrix; and P_r = energy expended on gamete production.

Secondary production for the somatic and shell parts for the 1988 and 1989 study periods was estimated using the increment summation method outlined by Rigler and Downing (1984). This method calculates production of cohorts as the sum of the change in biomass over specified time intervals.

The density of the population was estimated from a population census along each of three transects running perpendicular to the shore line. The number of *L. littorea* in every 5th m^2 were counted, and a random sample of 25 were measured for spire height. Biomass was determined monthly for each size class or cohort (from demographic analysis) in grams of ash-free dry weight (AFDW). This was done by interpolating the mean spire height of each cohort onto the graph of the log regression of the AFDW (somatic tissue and shell) on the log spire height of 50 individuals in the population at each sampling time. All regression equations were highly significant ($R^2 > 0.8$). This estimate of weight was then multiplied by the cohort density to give an estimate of biomass in $gAFDW/m^2$. Biomass determination formed the basis of production calculations. Biomass estimates were converted to energy units using the following conversion of Grahame (1973) of $24.7 \text{ kJ/mg}^1 \text{ AFDW}$.

The reproductive output was calculated directly for females using the direct method of Crisp (1984). *L. littorea* females, of a range of spire heights, were housed individually in plastic jars, and the spawn from each was collected (Chase and Thomas 1995b). Four samples of approximately 2,000 eggs were incinerated in a muffle furnace at 435°C for 3 h for ash-free determinations. Because there was little variation in the calculated weights, the mean of the samples were used in all calculations.

The reproductive output of males could not be calculated directly as in the females. Indirect analysis of fecundity was based on the mean gonad weight before and after spawning was known to have occurred (weight loss following spawning). For comparison purposes, the female reproductive output was also calculated using the indirect method (Crisp 1984). From May to November, 1989 samples of approximately 230 *L. littorea* were collected from Pendleton Island and taken to the laboratory, where they were maintained without food in running aerated seawater for 48 hours to allow the gut to clear (Grahame 1973). Histological analysis of the gonads of *L. littorea* in Pendleton Island revealed that the breeding season was between May and October, and spawning generally occurred between June and September (Chase and Thomas 1995a). Samples of approximately 100 each of males and females were killed by boiling for 1 minute. The gonadal region (including the penis and prostate in the males and the shell gland of the females) was dissected out and weighed before and after drying for 48 h at 60°C . In *L. littorea*, the gonadal tissue is found closely associated with the digestive system and could not be separated for individual measurement. It was assumed that by emptying the gut and consistently taking the same tissue for each sample, any difference in weight would be attributed to changes in the gonad. However, estimates of reproductive output may be more variable as a result of this approach. Samples were incinerated in a muffle furnace at 435°C for 3 h for ash-free determinations. Covariance analysis of gonad weight versus spire height was used to test for significant changes in gonad weight between sampling dates. This may indicate spawning. Further evidence of spawning was obtained through histological examination of the gonadal tissue (Chase and Thomas 1995a). The differences in gonad weight between these data was used in the calculation of

gamete production. Biomass estimates for gonadal tissue were converted to energy units using the conversion of Grahame (1973) of 26 kJ/mg¹ AFDW.

RESULTS

Demography 1988

Figure 2(a-c) are histograms of the population of *L. littorea* on Pendleton Island at peak reproductive times (i.e., settlement): May (Fig. 2a), July (Fig. 2b), and August (Fig. 2c) 1988. Summary of mean spire height (mm \pm SD) and density (number of individuals/m²) of each cohort are shown in Table 1. Cassie (1954) analysis revealed that the population was composed of four cohorts from 3 years in 1988. In May, the population was dominated by mature individuals (>13 mm), which represented 71% of the population. The percentage of mature individuals in the population declined throughout the season to 46 and 52% of the population in July and August, respectively, as a result of settlement of new cohorts and subsequent reduction of older individuals from the population.

Settlement on Pendleton Island seems to involve the recruitment of two distinct cohorts, cohorts 0+ in July (Fig. 2b) and 0++ in August (Fig. 2c). Two cohorts per year remained discernable until the end of the first year, after which only one cohort per year could be identified. This is because older individuals have a very slow growth rate as opposed to the young, faster growing cohorts (Williams 1964), resulting in a merging of the younger cohorts with the older. In 1988, the newly settled cohorts (0+ and 0++) comprised 22.8% of the population at the end of the breeding season.

Demography 1989

Figure 2(d-f) are histograms of the population at peak reproductive times in May (Fig. 2d), August (Fig. 2e), and October (Fig. 2f) 1989. Summary of mean spire height (mm \pm SD) and density (number of individuals/m²) of each cohort are in Table 1. Cassie (1954) analysis revealed that the population was composed of four cohorts from 3 years [1a and 1b, (0++ and 0+ of the previous year), 2, and 3]. A cohort four was detected in May and August, but comprised only 5 and 0.3% of the population in each month. In

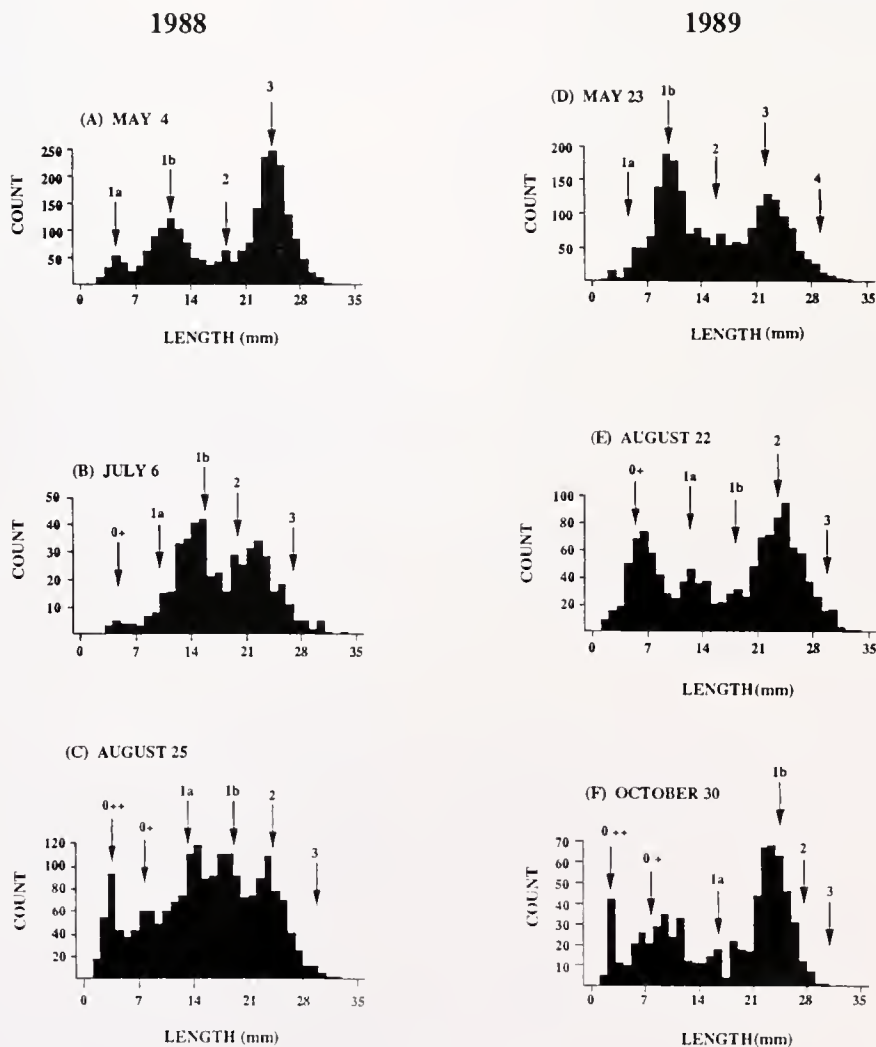


Figure 2. Length-frequency histograms of the *Littorina littorea* population at Pendleton Island at sampling intervals in 1988 (2 a-c) and 1989 (2 d-f). Arrows indicate the mean spire height (mm) of the component cohorts in the population. 1988: (A) May 4, (B) July 6, and 1989: (C) August 25, (D) May 23, (E) August 22, (F) October 30.

TABLE 1.

Summary of the mean spire height (mm \pm SD) and density (number of individuals/m²) of the component cohorts in the *Littorina littorea* population at Pendleton Island at sampling intervals in 1988 and 1989.

1988								
May			July			August		
Cohort	Mean Spire Height (mm) (\pm SD)	Density (#/m ²)	Cohort	Mean Spire Height (mm) (\pm SD)	Density (#/m ²)	Cohort	Mean Spire Height (mm) (\pm SD)	Density (#/m ²)
						0++	2.5 \pm 0.9	20
			0+	3.7 \pm 0.6	3	0+	8.5 \pm 1.6	4
1B	4.1 \pm 1.0	2	1B	10.0 \pm 2.4	1	1B	12.6 \pm 1.7	2
1A	10.6 \pm 2.3	1	1A	14.6 \pm 1.8	4	1A	17.2 \pm 1.3	4
2	17.2 \pm 2.6	7	2	19.4 \pm 0.9	6	2	22.5 \pm 2.2	18
3	24.2 \pm 2.2	18	3	26.4 \pm 2.0	19	3	26.0 \pm 0.8	4
1989								
May			August			October		
Cohort	Mean Spire Height (mm) (\pm SD)	Density (#/m ²)	Cohort	Mean Spire Height (mm) (\pm SD)	Density (#/m ²)	Cohort	Mean Spire Height (mm) (\pm SD)	Density (#/m ²)
						0++	2.5 \pm 0.9	73
			0+	4.9 \pm 1.9	12	0+	7.3 \pm 1.4	1
1B	4.1 \pm 1.2	2	1B	10.7 \pm 1.5	2	1B	12.8 \pm 2.6	2
1A	10.8 \pm 2.2	1	1A	17.1 \pm 1.5	8	1A	20.0 \pm 2.0	3
2	14.3 \pm 2.1	2	2	22.7 \pm 1.6	12	2	23.8 \pm 1.0	3
3	24.1 \pm 2.5	27	3	26.4 \pm 1.2	5	3	26.5 \pm 0.5	4
4	27.8 \pm 1.5	5						

May, the population was dominated by mature individuals (>13 mm), which represented 60% of the population. The percentage of mature individuals in the population declined throughout the season to 54 and 45% of the population in August and October, respectively, as a result of the settlement of new cohorts and the subsequent reduction in the older individuals (cohort 3: 34% in May to 2% in October) and the loss of cohort 4. Settlement of cohorts occurred in August (Fig. 2e) and October (Fig. 2f), 1989. Experimental data have shown that the differential timing may be the result of local temperature variations (Chase and Thomas 1995b). In 1989, the newly settled cohorts (0+ and 0++) comprised 31% of the population at the end of the breeding season.

Somatic and Shell Production

Table 2 contains the values calculated for production of somatic tissue (Pg) and shell (Ps), in kJ/m², for the 1988 and 1989 population of *L. littorea* on Pendleton Island. Somatic production (Pg) for the 1988 season was 70,265 kJ/m². Shell production (Ps) for the same time period was 16,859 kJ/m². An analysis of the production per cohort revealed the largest contribution was from the larger, hence older, cohorts 2 and 3. Production of these two cohorts represented 91.7% of Pg and 88.6% of Ps.

Somatic production (Pg) for the 1989 season was 87,764 kJ/m². Shell production (Ps) for the same time period was 8,974 kJ/m². Analysis revealed the majority of Pg was from cohorts 2 and 3 (74.5%); whereas, the majority of Ps was from cohorts 1 and 2 (88.8%). The decrease in Pg of cohort 4 and the negative Ps for cohorts 3 and 4 were probably a reflection of the decrease in density of each cohort (cohort 3: 1988, 18 individuals/m² to 4

individuals/m² in 1989; cohort 4: 5 individuals/m² in 1988 to 0 individuals/m² in 1989).

A two-way analysis of variance (ANOVA) on the mean somatic production (Pg) of each cohort, for the population in 1988 and 1989 revealed no significant year effect ($df = 1,3$; $F = 0.966$; $p > .5$); however, there were significant differences among cohorts ($df = 1,3$; $F = 25.95$; $p > .02$). Production of cohorts 0 and 1 was higher in 1989. A two-way analysis of variance on the mean shell production (Ps) of each cohort, for the population in 1988 and 1989 revealed no significant effect of year ($df = 1,3$; $F = 0.546$; $p > .5$) or cohort ($df = 1,3$; $F = 1.18$; $p > .5$).

Production of Gametes (Pr)

Female Reproductive Output

The mean (\pm SE) AFDW of four batches of approximately 2,000 eggs was $1.15 \times 10^{-6} \pm 0.2 \times 10^{-6}$ g AFDW per egg. Table 2 shows the production of gametes for females (Prf) using both direct and indirect methods of determination. Estimated Pr in 1989 using the direct method was 46,160 kJ/m². Pr was high at the onset of maturity (>13 mm, cohort 1) at 13,661 kJ/m², and in cohort 3 (22,038 kJ/m²) but decreased in the oldest cohort. This reduction in Pr is probably a reflection of the decrease in density of this cohort in the population. The estimated Prf in 1989 using the indirect calculation was 33,020 kJ/m². Production estimates using this method increased with the age of each cohort 3 (20,865 kJ/m²) and then decreased in cohort 4 (11,496 kJ/m²). A two-way ANOVA revealed that there was no significant difference effect of method ($df = 1,4$; $F = 0.72$; $p > .5$) or cohort ($df = 1,4$;

TABLE 2.

Estimated production (kJ/m^2) of shell (Ps, 1988 and 1989), somatic tissue (Pg, 1988 and 1989), and the production of gametes (Pr, 1989) of *Littorina littorea* on Pendleton Island.

Cohort	Production ($\text{KJ/m}^2/\text{y}^1$)								
	1988		1989						
	Pg	Ps	Pg	Ps	Prf (Indirect)	Prf (Direct)	Prm (Indirect)	Pr(m + f) (Total)	Pt
0	0.565	0.304	1.741	1.235	0	0	0	0	2.976
1	5.268	1.621	15.637	4.039	0.002	13.661	0.003	0.005	19.681
2	31.469	7.870	26.981	5.716	0.657	4.084	0.438	1.095	33.792
3	32.963	7.064	38.290	-0.962	20.865	22.038	15.740	36.606	73.933
4	—	—	5.115	-1.054	11.496	6.378	5.410	16.906	20.967
Total	70.265 (80.6%)	16.859 (19.4%)	87.764 (60.0%)	8.974 (5.9%)	33.020	46.160	21.591	54.114 (36.1%)	151.349

Percentage total production allocated to each component in parentheses (/).

Production gametes (Pr) presented for females (Prf) and males (Prm) separately and together [Pr(m + f)].

Prf calculated using both indirect and direct methods (Crisp 1984).

$F = 5.83$; $p > .1$). However, the indirect method seems to underestimate the Pr of the younger cohorts. Indirect estimates seem to be inappropriate for young cohorts, and fecundity estimates based on such will underestimate Pr, especially for the males.

Male Reproductive Output

The total annual production of male gametes (Prm) was calculated to be $21,591 \text{ kJ/m}^2$ (Table 2). Production increased to a maximum at cohort 3 ($15,740 \text{ kJ/m}^2$, 72.9% of the total Pr). A two-way ANOVA on Pr of male and females using the indirect calculated values revealed no significant effect of sex ($df = 1,4$; $F = 2.81$; $p > .2$); however, cohort was significant ($df = 1,4$; $F = 27.68$; $p > .01$). Pr for the older females (cohorts 2 and 3) was larger than that of the males. The total estimate of Pr for the *L. littorea* population (males and females) on Pendleton Island based on the indirect calculations of male and female reproductive output was $54,114 \text{ kJ/m}^2$.

Total Production

The total production for the breeding season in 1989, calculated as the sum of Ps, Pg, and Pr, was $151,349 \text{ kJ/m}^2$ (36.1% Pr, 60.0% Pg, and 5.9% Ps). Total production could not be calculated for the 1988 season, because no data were available on production allocated to reproduction. Figure 3 shows the percentages of production allocated to each of Ps, Pg, and Pr in each cohort. In the newly settled cohort (0) all of the production was allocated to either Ps (41.5%) or Pg (58.5%). The percentage of production allocated to Ps and Pg in each cohort decreased once maturity was reached (cohort 1). The percentage of the production allocated to Pr increased with age to age 4, with Pr comprising 76.8% of the production of that cohort.

DISCUSSION

Demography

The structure of the intertidal population of *L. littorea* on Pendleton Island changed seasonally as a result of recruitment and growth (Fig. 2). At the beginning of each of the 1988 and 1989 seasons, the population of *L. littorea* was dominated by mature individuals ($>13 \text{ mm}$). However, at the end of the season, the

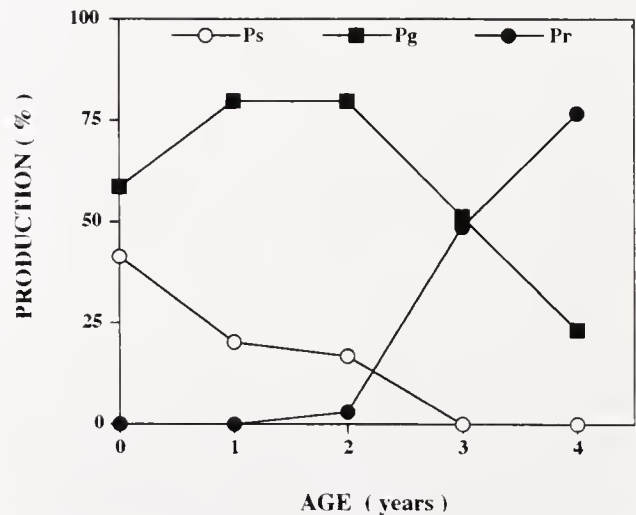


Figure 3. Percentage of total production in each age class that is allocated to shell growth (Ps), somatic tissue growth (Pg), and production of gametes (Pr) in 1989.

percentages of the population composed of immature and mature individuals were almost equal (% immature:% mature: 48:52 in 1988 and 45:55 in 1989). Recruitment of *L. littorea* was temporally variable but consisted of two pulses per year, in July and August 1988 and in August and October 1989. The newly settled cohorts comprised 23 and 31% of the population at the end of each season in 1988 and 1989. Recruitment was much higher on Pendleton Island than recorded in other studies of *L. littorea* (8.4% Gardner and Thomas 1987b at Welch's Cove, Bay of Fundy; 1.6% Lambert and Farley 1968 at Ketch Harbour, Nova Scotia; and 0.4–4.4% Smith and Newell 1955 at Whitsable, England). Larger recruitment densities may be the result of the existence of two pulses of recruitment per year. In addition, *L. littorea* on Pendleton Island were observed to recruit at high shore levels only (80% tidal level) (Chase and Thomas 1995a). Low recruitment densities in the studies of Gardner and Thomas (1987b) and Lambert and Farley (1968) were attributed to recruitment from subtidal regions that was not accounted for in fall estimates of recruitment density.

Production

Secondary production estimated for *L. littorea* on Pendleton Island in 1988 was measured as the production of somatic tissue and shell. Reproductive output was not included. In 1989, gamete production comprised 36.1% of the total production; thus, any calculations for *L. littorea* in 1988 were underestimated. Comparison of the somatic and shell production estimates in 1988 and 1989 revealed that there was no difference between years, despite a difference in the duration of the sampling season. In 1988, production was measured from early May to late August as compared to early May to late October in 1989, a difference of approximately 8 weeks. A breakdown of production in 1989 into two time periods: May to late August and late August to late October; however, revealed that the majority of shell (64.7%) and all of the somatic production (100%) occurred in the May to late August time period.

Comparisons of total secondary production values of *L. littorea* are difficult, because no studies have examined all components of total production (somatic tissue, shell growth, and reproductive output). There are, however, studies where somatic tissue and/or gamete production have been measured, so some comparisons are possible. Gardner and Thomas (1987b) measured somatic tissue production for a population of *L. littorea* at Welch's Cove in the Bay of Fundy. Their calculated value was approximately 3X larger than our figures; although the populations at each location were very different, which may prevent comparisons between the studies. The study of Gardner and Thomas (1987b) was restricted to a small portion of the intertidal zone and had much higher densities than our study (657 to 934 individuals/m² versus 28 to 86 individuals/m²). In contrast, our study examined the entire intertidal zone.

Grahame (1973) calculated somatic production and gamete production of a population of *L. littorea* at Anglesey, Wales. Total production of these two measures was 852.51 kJ/m² [somatic production = 629.510 kJ/m² (138 kcal/m²) and gamete production = 223 kJ/m² (46.7 kcal/m²)]. The estimate of gamete production in this study was only 54 kJ/m², only 24% of that reported by Grahame (1973). The population in that study, however, contained many larger individuals (spire height > 25 mm). On Pendleton Island in 1989, the percentage of the population comprising individuals with a spire height > 25 mm was less than 3%. Because fecundity increases with spire height in *L. littorea* (Grahame 1973, Hughes and Roberts 1980, Chase and Thomas 1995b), it would be expected that we would observe a larger Pr for a population composed of larger, more fecund individuals.

No estimate of the production of the shell matrix for *L. littorea* was found in the literature. Values from other studies indicate that shell production in bivalves is usually <5% of total production. For *L. littorea*, the organic component of the shell represented 6% of the total production in 1989. As such, it seems that, for *L. littorea*, the majority of the secondary production, was attributed to the production of somatic tissue and gametes.

Considering such a large amount of energy is required for the production of gametes, it would be expected that there would be a close coupling between the reproductive cycle and energy available for growth. Our data are in keeping with the general observation that animals devote a greater share of the production to reproduction as they age (Fig. 3). However, the proportion of total production allocated to reproduction in the *L. littorea* population on Pendleton Island was higher as compared to other studies.

Hughes and Roberts (1980) examined the proportion of total

production allocated to reproduction (Pr/Pr + Pg) for different age classes of four Littorinid species, including *L. littorea*. They observed, for all species, that the proportion of total production allocated to reproduction increased linearly during a phase of rapid growth, but began to level off abruptly toward its asymptotic value of 80 to 100% (Hughes and Roberts 1980). For *L. littorea*, specifically, the proportion allocated to reproduction increased from 0 to 100% in 9 years, with the asymptote occurring at age 5 (Hughes and Roberts 1980). In this study, only four cohorts were discernable, either because older individuals were not present in the population, or they were so few in numbers so as not detectable using this method of cohort determination. Over the 4 years, however, the proportion of total production allocated to reproduction increased linearly once maturity was reached (cohort 1). For an age 4 individual, the proportion of total production allocated to reproduction (Pr) in *L. littorea* was approximately 60% in the study of Hughes and Roberts (1980). In this study, Pr in an age 4 individual was 76.8%.

Alternative estimates of the proportional allocation to reproduction include the ratio of gamete to somatic production (Pr/Pg). In this study, Pr/Pg was 61.7%. This estimate is high as compared to studies on other gastropods, including *L. littorea*, where Pr/Pg was calculated; 34% (*L. littorea*, Grahame 1973), 30 to 40% (*Lacuna vincta*, Grahame 1982), 25% (*Lacuna pallidula*, Grahame 1982), and 11.7% (*Fissurella barbadensis*, Hughes 1971).

Developing hypotheses to explain the higher allocation to reproduction (Pr) observed in this study is partly hampered by only 1 year of measurement. In many studies, the larger Pr may reflect older populations, because Pr generally increases with the age of an individual (Hughes and Roberts 1980, this study). The population monitored in this study was made up of young individuals (only four cohorts detected in each year). A high allocation to reproduction in such young animals might be expected if this population is subject to high mortality and lowered life expectancy. This reduced life expectancy may be the result of environmental conditions. The population of *L. littorea* on Pendleton Island is situated in a passageway where there is a very strong current. Average surface currents 3 hours before and after low tide generally exceed 1.3 m/s¹, bottom currents have been measured at 0.47 to 0.60 m/s¹ (Thomas et al. 1990). Many studies examining the effect of wave exposure on the population energetics of gastropods have found greater mortality and a larger amount of energy devoted to reproduction (e.g., Hughes and Roberts 1980; Hart and Begon 1982; Etter 1989). In addition to mortality caused by environmental conditions, it was found that at least 25% of the larger animals examined had parasitic infestations of the digenetic trematode larvae, *Cryptocotyle lingua* (Crepling) (Chase and Thomas 1995b). Similar infestations have resulted in the destruction of the digestive gland, castration, and change in the migration pattern (Fretter and Graham 1962; Lambert and Farley 1968). Such infestations may explain the absence of larger/older individuals on this shore.

SUMMARY

The results of this study revealed that estimates of population production of *L. littorea* were much lower than values reported in the literature. Rates of secondary production are known to vary widely in nature and are affected by a variety of biotic and abiotic characteristics of the environment (Plante and Downing 1989). Such variation in production may be the result of independent or

combined effects of annual differences in such environmental factors as water temperature and/or food supply, variation in density, age structure, and allocation to growth and reproduction. Density and age structure have been proposed as factors causing the lower somatic and gamete production values observed in this study. However, despite lower estimates of production, the proportion of total production allocated to reproduction (Pr) measured as both the proportion of total production ($(Pr/Pg + Pr + Ps)$) and somatic production ((Pr/Pg)) was higher in this study by approximately 20% as compared to estimates for *L. littorea* in the literature.

The Bay of Fundy exhibits a great diversity of marine species, a combination of the influx of larvae, propagules, and adults from the Labrador Current and the Gulf of Marine waters, and the high productivity as a result of the vigorous tidal mixing (Thomas et al. 1990). Prior research on the population of *L. littorea* in Pendleton Passage revealed high growth rates, large recruitment densities, and high reproductive outputs (Chase and Thomas 1995a,b). However, analysis of the demography, production, and resource allocation of the population of *L. littorea* in Pendleton Passage seems

to be more characteristic of an environment with very harsh conditions; that is, fewer older individuals, faster turnover rates, and lower total production, with a greater proportion of total production allocated to reproduction. It is likely that the high tidal currents are a major influence on the population of *L. littorea* in Pendleton Passage. However, additional information will have to be gathered before we can speculate on the effects of high tidal energy on this population of *L. littorea*.

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IDENTIFICATION OF STOCKS OF THE EXPLOITED LIMPETS *PATELLA ASPERA* AND *P. CANDEI* AT MADEIRA ARCHIPELAGO BY ALLOZYME ELECTROPHORESIS

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ABSTRACT Allozyme electrophoresis was used to investigate stock integrity of *Patella aspera* (= *P. ulyssiponensis*) and *Patella candei* (Mollusca, Patellogastropoda) from the Madeira Archipelago. Samples from the north of the island of Madeira (Porto Moniz) and the north of Deserta Grande were taken for both species, and a sample of *P. aspera* was also taken from the south of the island of Madeira (Caniço). Twenty-one putative loci were resolved and analyzed for both species. Significant differences in allele frequencies were found between locations in each species, suggesting the presence of different genetic stocks. *P. candei* was found to be slightly structured with a small standardized variance in allele frequencies between samples from Porto Moniz and Deserta Grande ($F_{ST} = 0.036$); whereas, *P. aspera* was highly structured, with an F_{ST} value over all subpopulations of 0.157. Our results indicate more genetic interchange between the populations from the north of Madeira and Deserta Grande than between north and south Madeira. Our findings are consistent with surface water-mass circulation patterns from northwest to southeast along the Madeira Archipelago, determined mainly by the Canary Current. Species with low dispersal and a high degree of spatial genetic structuring among subpopulations are more susceptible to collapse caused by overfishing than those with high dispersal capabilities; therefore, the individual management and control of these biological units is recommended.

KEY WORDS: Mollusca, patellogastropoda, *Patella*, population genetics, stocks, allozymes

INTRODUCTION

The stock concept was first described by Larkin (1972) as: "a population of organisms sharing a common gene pool, that is discrete to warrant consideration as a self-perpetuating system that can be managed." This concept, primarily applied to fish species, has been widely used with different connotations by the fisheries community, politicians, and biologists. Stock, as a management unit, may also be defined as the extent of a population or mixed populations over which a fishing activity occurs. Therefore, it may comprise more than one biological stock or subpopulation (Hedgecock 1986).

In the 1970s, allozyme electrophoresis emerged as a powerful tool to recognize biological stocks by means of genetic information (Gall 1986, Hedgecock 1986, Utter 1986, Utter 1991, Shepherd and Brown 1992, Avise 1994). Once the genetic structure of an exploited species is understood, the fishery can be regulated so that harvesting of each subpopulation can be managed and controlled individually (Allendorf et al. 1988). However, despite its importance, the "stock-composition" strategy has been practiced rarely in fisheries management (Pella and Milner 1988).

P. aspera Röding, 1798 (= *P. ulyssiponensis* Gmelin, 1791) and *P. candei* d'Orbigny, 1840 are two patellogastropod limpets distributed throughout the Macaronesian Archipelagos, where they represent an important food resource. In recent years, they have been intensively exploited for subsistence and for commercial purposes. In the Azores, both species have been declining for many years (Martins et al. 1987, Menezes 1991, Côrte-Real et al. 1996).

Recent works on the population genetics of these limpets, using allozyme electrophoresis (Côrte-Real 1992, Côrte-Real et al. 1996, Weber et al. in preparation), have shown high macroscale structuring of their populations. The aim of the present work is to study the structuring of these species within the Madeira Archipelago, to

find out if there are different biological stocks. This work forms part of a fisheries management strategy by the regional government.

MATERIALS AND METHODS

Sampling Sites

Samples of *Patella aspera* and *Patella candei* were taken from the Madeira Archipelago (Fig. 1). Samples of both species were taken in July 1994 from Porto Moniz (north Madeira) and from the north of Deserta Grande in March 1995. An additional sample of *Patella aspera* was taken from Caniço (south Madeira) in July 1994. Locations in Madeira were chosen from those points where human activity is concentrated. Sample sizes (n) are specified in Tables 2 and 3. All samples were transported live to the Port Erin Marine Laboratory, where they were frozen at -78°C until required for electrophoresis.

Electrophoresis

Homogenates were prepared by macerating foot muscle in buffer Tris-HCl, pH 8.0, and then centrifuged at 10,000 rpm for 5 min. Supernatants were used afterward for electrophoresis. Standard horizontal 12.5% starch gel (Sigma-Aldrich Co. Ltd.) electrophoresis was carried out using the following buffer systems: (I) Tris-citrate, pH 8.0 (Siciliano and Shaw, 1976) and (II) Tris-citrate-EDTA, pH 7.0 (Ayala et al. 1972); (III) Discontinuous Tris-citrate-borate, pH 8.2–8.7 (Poulík 1957); (IV) Tris-citrate-borate-LiOH, pH 8.26–8.31 (Redfield and Salini 1980); (V) Tris-borate EDTA, pH 9.0 (Ayala et al. 1974). Specific staining procedures for the enzymes analyzed (Table 1) followed the techniques of Brewer (1970), Shaw and Prasad (1970), Harris and Hopkinson (1976) and Murphy et al. (1990).

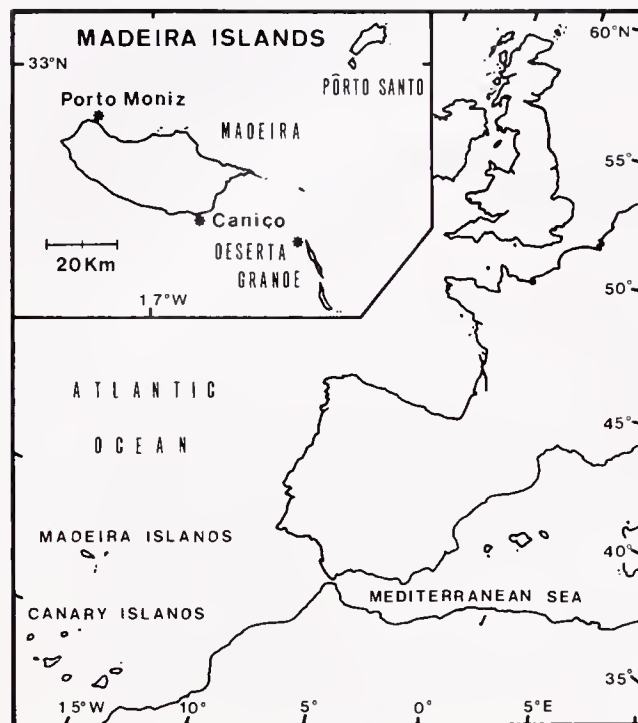


Figure 1. (*) Sampling sites for *P. candei* and *P. aspera* (see text for details).

Allele frequencies, mean expected heterozygosity, Nei's (1978) genetic identities, and χ^2 tests for homogeneity in allele frequencies were calculated using the statistical package BIOSYS-1 (Swofford and Selander 1981). For those individual tests of heterogeneity that showed $p < .05$, contingency tables were checked using G (log-likelihood ratio)-test with pooling when more than two alleles, and expected frequencies less than five were observed. Yate's correction was also applied for those 2×2 tables, p-values after G-test were reported in Tables 2 and 3 only when they were

notably different from those obtained by the common χ^2 . Unbiased inbreeding coefficients, F_{IS} and F_{IT} (Weir and Cockerham 1984) and the genetic variance between populations (F_{ST}), with their respective 95% confidence intervals, estimated from a bootstrap procedure of 15,000 resamplings, were obtained by the FSTAT Program, version 1.2 (Goudet 1995). Numbers of migrants between populations per generation (n_{em}) were calculated by using Slatkin's (1993) formula: $n_{em} = (1/F_{ST} - 1)/4$. Loci were numbered according to increasing anodal mobility.

RESULTS

Twenty one loci were resolved for both species. *Est-D* was only resolved for *P. candei* and *Ldh-1* only for *P. aspera*. Allele frequencies, variability measures, F-statistics, and results of the χ^2 test for the homogeneity in allele frequencies are summarized in Tables 2 and 3 for *P. candei* and *P. aspera*, respectively.

Variability

The locus *Gludh* was fixed for the same allele in all populations of both species, and *Mdh-1* was fixed in all the populations of *P. aspera*. High levels of polymorphism (76–85.7%) and heterozygosity were registered for both species. *P. aspera* populations showed higher values of heterozygosity (all over 21%) than *P. candei* (around 10 to 12%). Mean number of alleles per locus was also higher in *P. aspera* (4.4) than *P. candei* (2.7).

Differentiation among Subpopulations

Both species showed significant differentiation in allele frequencies between locations (see χ^2 tests in Tables 2 and 3).

P. candei Subpopulations

The genetic identity (Nei, 1978) between *P. candei* samples from Porto Moniz and Deserta Grande was 0.995. An F_{ST} value of 0.036 was found for the analyzed *P. candei* samples, indicating the subdivision of the total population. The bootstrap 95% confidence interval showed that the F_{ST} value was close to but different from

TABLE 1.
Name, E.C. number, abbreviation, buffer system and number of loci for each enzyme analyzed

Enzyme Name	E.C. Number*	Abbreviation	Number of loci	Buffer System
Aspartate aminotransferase	2.6.1.1	AAT	2	III
Esterase	3.1.1.-	EST	2	IV
Esterase-D	3.1.-	EST-D	1	III
Fructose-biphosphate aldolase	4.1.2.13	FBALD	1	V
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH	1	V
Glutamate dehydrogenase	1.4.1.-	GLUDH	1	V
Glucose-6-phosphate isomerase	5.3.1.9	GPI	1	I
Isocitrate dehydrogenase (NADP+)	1.1.1.42	IDHP	2	I
L-Lactate dehydrogenase	1.1.1.27	LDH	2	II
Malate dehydrogenase	1.1.1.37	MDH	2	IV
Malic Enzyme (NADP+)	1.1.1.40	MEP	2	I
Mannose-6-phosphate isomerase	5.3.1.8	MPI	1	IV
Phosphoglucomutase	5.4.2.2	PGM	1	II
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	1	I
Purine-nucleoside phosphorilase	2.4.2.1	PNP	1	I
Superoxide dismutase	1.15.1.1	SOD	2	III

* IUBNC(1984); Shaklee et al. (1990).

TABLE 2.

Patella candei; allele frequencies, F-statistics per locus, and over-all loci (with 95% confidence intervals in parentheses obtained by Bootstrap procedure) and contingency χ^2 test for the homogeneity in the allele frequencies for each locus and over-all loci.

Locus	Allele	Allele frequencies		F statistics per locus			Homogeneity Test	
		DES (n = 50)	MNZ (n = 50)	F _{IS}	F _{IT}	F _{ST}	χ^2	p
<i>Aat-1</i>	A	0.150	0.020	-0.010	0.085	0.094	10.865	0.00191†
	B	0.850	0.980					
	All							
<i>Aat-2</i>	A	1.000	0.950	-0.043	-0.000	0.041	5.128	0.05353†
	B	0.000	0.050					
	All							
<i>Est-1</i>	A	0.110	0.110	0.171	0.167	-0.005	1.473	0.47878
	B	0.770	0.820					
	C	0.120	0.070					
<i>Est-2</i>	A	1.000	0.980	-0.010	0.000	0.010	2.020	0.15522
	B	0.000	0.020					
	All							
<i>Est-D</i>	A	0.080	0.060	0.152	0.174	0.026	2.083	0.14892
	B	0.920	0.940					
	All							
<i>Fbald</i>	A	0.960	1.000	-0.032	-0.000	0.031	4.082	0.11434†
	B	0.040	0.000					
	All							
<i>Gapdh</i>	A	0.000	0.010	0.000	0.000	0.000	1.005	0.31610
	B	1.000	0.990					
	All							
<i>Gpi</i>	A	0.020	0.010	-0.034	-0.019	0.014	4.464	0.21550
	B	0.930	0.980					
	C	0.040	0.000					
	D	0.010	0.010					
	All							
<i>Idhp-1</i>	A	0.010	0.010	-0.003	-0.010	-0.007	1.005	0.60499
	B	0.990	0.980					
	C	0.000	0.010					
	All							
<i>Idhp-2</i>	A	0.030	0.030	-0.081	0.149	0.074	10.237	0.01755†
	B	0.670	0.450					
	C	0.290	0.500					
	D	0.010	0.020					
	All							
<i>Ldh-2</i>	A	0.060	0.050	0.241	0.232	-0.013	0.424	0.80887
	B	0.010	0.020					
	C	0.930	0.930					
	All							
<i>Mdh-1</i>	A	0.000	0.010	0.000	0.000	0.000	1.005	0.31610
	B	1.000	0.990					
	All							
<i>Mdh-2</i>	A	0.170	0.000	-0.196	-0.000	0.164	19.780	0.00005*
	B	0.820	1.000					
	C	0.010	0.000					
	All							
<i>Mep-1</i>	A	0.010	0.010	-0.000	-0.010	-0.010	0.000	0.99835
	B	0.990	0.990					
	All							
<i>Mep-2</i>	A	0.010	0.010	-0.039	-0.027	0.011	5.932	0.20426
	B	0.970	0.920					
	C	0.010	0.040					
	D	0.010	0.000					
	E	0.000	0.030					
	All							

continued on next page

TABLE 2.
continued

Locus	Allele	Allele frequencies		F statistics per locus			Homogeneity Test	
		DES (n = 50)	MNZ (n = 50)	F _{IS}	F _{IT}	F _{ST}	χ^2	p
<i>Mpi</i>	A	0.020	0.010	0.192	0.187	-0.006	2.338	0.31061
	B	0.980	0.970					
	C	0.000	0.020					
	All							
<i>Pgdh</i>	A	0.000	0.020	-0.024	-0.014	0.011	3.082	0.21412
	B	0.010	0.030					
	C	0.990	0.950					
	All							
<i>Pgm</i>	A	0.000	0.020	0.109	0.120	0.012	4.752	0.09294
	B	0.210	0.120					
	C	0.790	0.860					
	All							
<i>Pnp</i>	A	0.230	0.170	0.130	0.130	-0.000	1.125	0.28885
	B	0.770	0.830					
	All							
<i>Sod-2</i>	A	0.010	0.020	-0.007	-0.014	-0.007	0.338	0.56075
	B	0.990	0.980					
	All							
Overall loci	Ho ^a	0.117	0.105	0.071 (0.006–0.113)	0.105 (0.057–0.132)	0.036 (0.006–0.065)	81.240	0.00001*
	He ^b	0.126	0.109					
	P _{0.99} ^c	76.2	85.7					

^a Mean observed heterozygosity (direct-count).^b Mean unbiased heterozygosity based on Hardy–Weinberg expectation (Nei 1978).^c Percentage of polymorphic loci: a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.* Significant at $\alpha = 0.05$ after a Bonferroni procedure for multiple tests was applied ($\alpha' = \alpha/1 + k-i$; for k = number of tests, and i = rank of the values of p when ordered from the smallest to the largest; when $p_i \leq \alpha'$, then the corresponding test indicates significance at the "table-wide" α level (Rice, 1989).

† P after G test and Yate's correction.

Key: F_{IS} Mean heterozygote deficiency within populations; F_{IT} Heterozygote deficiency in the total population; F_{ST} Degree of differentiation between subpopulations; MNZ: Pto. Moniz; DES: Deserta Grande; (n) Sample size.

zero (Table 2). The results of the χ^2 test corroborated the presence of different subpopulations although with only slight differences.

The significant differentiation found in *P. candei* between Porto Moniz and Deserta Grande was largely a result of the variation in allele frequencies of two loci: *Aat-1* and *Mdh-2* (Table 2). These showed a geographic pattern in allele frequencies, with a tendency toward the fixation of the most common allele at Deserta Grande Island (see Fig. 2), with a resulting loss of genetic variability at Deserta Grande (see measures of genetic variability in Table 2). The number of migrants estimated between these subpopulations was 6.7 individuals per generation. F_{IS} values were high for various loci indicating a tendency of heterozygote deficit (see Table 2).

P. aspera Subpopulations

The genetic identities and distances between samples of *P. aspera* are shown in Table 4. The identity values ranged from 0.930 to 0.959 (see also UPGMA dendrogram in Fig. 4). The total F_{ST} estimated over-all samples was 0.156 (0.026–0.307), and 0.126 (0.013–0.262) between the most closely related locations (Canico and Deserta Grande), both values being different from zero. The estimated numbers of migrants obtained from the F_{ST} values are 1.7 individuals per generation between Canico and De-

serta Grande, 1.1 between Porto Moniz and Canico, and 1.5 between Porto Moniz and Deserta Grande.

A χ^2 test applied to over-all samples was highly significant, showing that seven of the 21 loci analyzed were responsible for the genetic differentiation among populations (see Table 3). Even between the most closely related pair of samples (Canico and Deserta Grande), there was highly significant differentiation in allele frequencies ($\chi^2 = 256.50$; $p < 10^{-5}$), which was mainly attributable to the contributions of 4 loci: *Est-1* ($p < 10^{-5}$), *Aat-1* ($p < 10^{-5}$), *Est-1* ($p < 10^{-4}$), and *Idhp-2* ($p < 10^{-4}$).

It was possible to detect geographic patterns in allele frequencies (Fig. 3) as in *P. candei*. Figure 3 shows the six loci that displayed the most significant differentiation between subpopulations. We could distinguish three different patterns. The first, detected only at the *Me-2* locus, showed that the less common allele of Porto Moniz, "B," became the most common one in Canico and Deserta Grande subpopulations. As a second geographic pattern, it was possible to detect a cline in three loci, *Aat-1*, *Est-1*, and *Sod-2*. At the *Aat-1* locus, the allele "C" of Porto Moniz increased its frequency from 23 to 36% in Canico and to 75% in Deserta Grande; at the *Est-1* locus, the most common allele "B" in Porto Moniz, also increased its frequency from 62 to 85% in Canico and to 87% in Deserta Grande; and at the *Sod-2* locus, the most com-

TABLE 3.

Patella aspera; allele frequencies, F-statistics per locus, and over-all loci (with 95% confidence intervals, between brackets, obtained by bootstrap procedure) and contingency χ^2 test for the homogeneity in the allele frequencies for each locus and over-all loci.

Locus	Allele	Allele frequencies			F statistics per locus			Homogeneity Test	
		MNZ (n = 100)	CNC (n = 49)	DES (n = 50)	F _{IS}	F _{IT}	F _{ST}	χ^2	p
<i>Aat-1</i>	A	0.025	0.000	0.040	0.159	0.382	0.265	83.914	0.00000*
	B	0.005	0.000	0.000					
	C	0.230	0.357	0.750					
	D	0.740	0.643	0.210					
	All								
<i>Aat-2</i>	A	0.005	0.000	0.000	0.077	0.077	0.000	6.924	0.54490
	B	0.080	0.031	0.090					
	C	0.790	0.867	0.780					
	D	0.115	0.102	0.130					
	E	0.010	0.000	0.000					
<i>Est-1</i>	All				0.262	0.316	0.073	55.329	0.00000*
	A	0.115	0.010	0.130					
	B	0.625	0.847	0.870					
	C	0.115	0.133	0.000					
	D	0.145	0.010	0.000					
<i>Est-2</i>	All				0.437	0.652	0.381	139.439	0.00000*
	A	0.045	0.000	0.030					
	B	0.790	0.306	0.940					
	C	0.155	0.694	0.030					
	D	0.010	0.000	0.000					
<i>Fbald</i>	All				0.137	0.136	-0.001	26.155	0.00021
	A	0.000	0.082	0.000					
	B	0.250	0.214	0.230					
	C	0.745	0.704	0.770					
	D	0.005	0.000	0.000					
<i>Gapdh</i>	All				0.126	0.118	-0.008	0.172	0.17660
	A	0.250	0.265	0.240					
	B	0.750	0.735	0.760					
	All								
	A	0.005	0.010	0.020					
<i>Gpi</i>	B	0.005	0.000	0.000	0.015	0.013	-0.002	13.218	0.35337
	C	0.895	0.929	0.910					
	D	0.025	0.000	0.010					
	E	0.000	0.000	0.020					
	F	0.065	0.061	0.040					
<i>Idhp-1</i>	G	0.005	0.000	0.000	-0.006	-0.007	-0.002	2.988	0.81041
	All								
	A	0.010	0.000	0.010					
	B	0.980	1.000	0.990					
	C	0.005	0.000	0.000					
<i>Idhp-2</i>	D	0.005	0.000	0.000	0.197	0.306	0.136	47.490	0.00000*
	All								
	A	0.010	0.000	0.000					
	B	0.010	0.010	0.040					
	C	0.010	0.020	0.010					
<i>Ldh-1</i>	D	0.000	0.000	0.010	-0.019	-0.014	0.006	7.110	0.52481
	E	0.670	0.969	0.760					
	F	0.300	0.000	0.180					
	All								
	A	0.010	0.000	0.000					
<i>Ldh-2</i>	B	0.955	0.980	1.000	0.282	0.285	0.004	17.992	0.07090†
	C	0.025	0.10	0.000					
	D	0.005	0.010	0.000					
	E	0.005	0.000	0.000					
	All								
	A	0.045	0.061	0.050	0.282	0.285	0.004	17.992	0.02128
	B	0.260	0.276	0.220					
	C	0.595	0.643	0.730					
	D	0.070	0.010	0.000					
	E	0.030	0.010	0.000					
	All								
	All								

continued on next page

TABLE 3.
continued

Locus	Allele	Allele frequencies			F statistics per locus			Homogeneity Test	
		MNZ (n = 100)	CNC (n = 49)	DES (n = 50)	F _{IS}	F _{IT}	F _{ST}	χ^2	p
<i>Mdh-2</i>	A	0.005	0.000	0.010	0.316	0.310	-0.009	1.270	0.86643
	B	0.965	0.969	0.970					
	C	0.030	0.031	0.020					
	All								
<i>Mep-1</i>	A	0.010	0.082	0.030	0.109	0.139	0.034	10.711	0.00799†
	B	0.990	0.918	0.970					
	All								
<i>Mep-2</i>	A	0.000	0.010	0.080	0.048	0.702	0.688	293.090	0.00000*
	B	0.075	0.929	0.810					
	C	0.895	0.061	0.110					
	D	0.025	0.000	0.000					
	E	0.005	0.000	0.000					
	All								
<i>Mpi</i>	A	0.000	0.102	0.030	0.250	0.307	0.075	21.705	0.00002*
	B	1.000	0.898	0.970					
	All								
<i>Pgdh</i>	A	0.005	0.000	0.020	0.111	0.128	0.020	9.249	0.05518
	B	0.040	0.031	0.100					
	C	0.955	0.969	0.880					
	All								
<i>Pgm</i>	A	0.015	0.000	0.000	0.070	0.066	-0.004	10.859	0.36858
	B	0.185	0.133	0.210					
	C	0.020	0.000	0.010					
	D	0.250	0.306	0.280					
	E	0.515	0.531	0.500					
	F	0.015	0.031	0.000					
	All								
<i>Pnp</i>	A	0.005	0.000	0.000	-0.051	-0.008	0.042	28.109	0.00173*
	B	0.000	0.000	0.020					
	C	0.005	0.000	0.070					
	D	0.980	0.990	0.900					
	E	0.010	0.010	0.000					
	F	0.000	0.000	0.010					
	All								
<i>Sod-2</i>	A	0.195	0.255	0.120	0.055	0.074	0.020	40.258	0.00002
	B	0.090	0.000	0.000					
	C	0.660	0.745	0.790					
	D	0.010	0.000	0.050					
	E	0.025	0.000	0.040					
	F	0.020	0.000	0.000					
	All								
Over-all loci	Ho ^a	0.228	0.176	0.181	0.155 (0.100–0.214)	0.287 (0.154–0.423)	0.156 (0.026–0.307)	815.981	0.00000*
	He ^b	0.254	0.217	0.224					
	P _{0.99} ^c	85.7	85.7	85.7					

^a Mean observed heterozygosity (direct-count).^b Mean unbiased heterozygosity based on Hardy–Weinberg expectation (Nei 1978).^c Percentage of polymorphic loci: a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.* Significant at $\alpha = 0.05$ after a Bonferroni procedure for multiple tests was applied (see Table 2).

† P after G test and Yate's correction.

Key: F_{IS} Mean heterozygote deficiency within populations; F_{IT} heterozygote deficiency in the total population; F_{ST} degree of differentiation between subpopulations; MNZ: Pto. Moniz; CNC: Caniço; DES: Deserta Grande; (n) sample size.

mon allele "C" in Porto Moniz also increased its frequency in the same geographic direction, from 65 to 74% and to 79%. Finally, the third pattern was mainly characterised by *Est-2* and *Idhp-2* loci, where the frequency patterns are more similar between Porto

Moniz and Deserta Grande than to the Caniço subpopulation. This last pattern results in the greater genetic identity found between Porto Moniz and Deserta Grande samples than between the former and the Caniço subpopulation (see Table 4). F_{IS} values were even

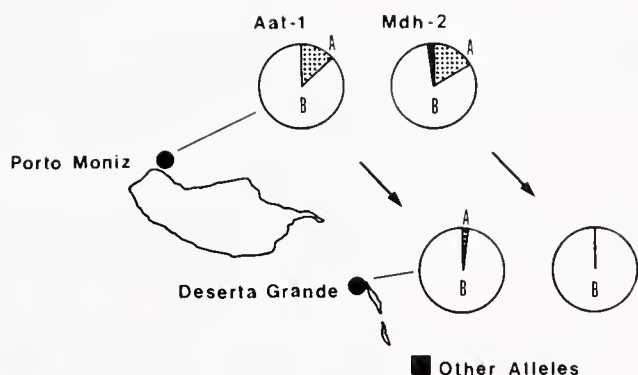


Figure 2. *P. candei*; geographic pattern of allele frequencies along Madeira archipelago at the *Aat-1* and *Mdh-2* loci.

higher than in *P. candei*, indicating a higher tendency toward heterozygote deficit with a mean over loci of 0.155 (see Table 3).

DISCUSSION

The mean observed heterozygosity obtained over all subpopulations of *P. candei* (0.111) is very close to the mean obtained over subpopulations of the same species (0.117) by Côté-Real et al. (1996). The mean value of heterozygosity over the populations of *P. aspera* at the Madeira Archipelago (0.195) is higher than the value (0.137) obtained for the Macaronesian Islands by Côté-Real (1992). Differences found between these values may be attributable to different number of loci analyzed and the different number of populations.

F_{IS} values were high in both species, indicating an heterozygote deficit. Excess of homozygotes is not uncommon in mollusks, being already found in *P. candei* and *P. caerulea* by Côté-Real et al. (1996). It has been explained by the presence of null alleles, inbreeding, negative heterosis, aneuploidy, and population mixing (see further references in Côté-Real et al. 1996). However, we do not have enough evidence to establish which is the case for explaining *P. candei* and *P. aspera* heterozygote deficit.

Our results showed that both species are structured in the Madeiran Archipelago. Although *P. candei* was slightly structured, maintaining levels of gene flow of about 6.7 migrants per generation between Porto Moniz and the north of Deserta Grande, *P. aspera* was highly structured, with a gene flow of only 1.5 individual per generation between the same localities, 1.1 between the

north (Porto Moniz) and south (Canico) of Madeira, and 1.7 between Canico and the north of Deserta Grande.

The length of the pelagic phase during larval development of *P. aspera* and *P. candei* is not known. Nevertheless, a larval dispersal phase of about 4 days after fertilization has been described for *P. vulgata* and *P. caerulea* (Dodd 1956). From the 4th day after fertilization the veliger starts to become more sedentary until becoming completely benthic after metamorphosis, around the 9th day after fertilization (Dodd 1956). A dispersal time of 4 to 9 days suggests restricted dispersal capabilities.

Patella vulgata showed levels of structuring ($F_{ST} = 0.027$) at a small and large geographic scale in the northeast of England and in south Wales (Hurst and Skibinski 1995) similar to those of *P. candei* in the Madeira Archipelago. Similar lengths of planktonic larval life have been described for the highly structured populations of *Tridacna gigas*, suggesting that those lengths of larval dispersal may not be sufficient to allow dispersal over large stretches of the ocean (Benzie and Williams 1995).

When the dispersal capability of a species is considerably less than its geographic range, genetic differences between subpopulations should increase with the distance separating them (Slatkin 1987, Hellberg 1994). The pelagic larvae of *P. aspera* and *P. candei* would have to travel at least 174 nautical miles from Porto Moniz to reach the north of Deserta Grande, approximately 177 nautical miles to reach Canico, but to cross from Canico to the north of Deserta Grande would have to travel only 16 nautical miles. The surface water circulation along the Madeira Archipelago is mainly caused by the Canary Current, which flows northwest to southeast, at around 0.72 km/h (Lalli and Parsons 1995). Pelagic larvae carried by this current would have to travel at least 18 days from the northwest of Madeira Island (Porto Moniz) to the southeast in direction to Deserta Grande to reach the north of this island. This period of time for limpet larvae to be in the plankton seems too long to allow them to travel from one island to the other before metamorphosis occurs. Nevertheless, more rapid transport could occur during storms, and, perhaps larvae can extend their pelagic phase for a time when no suitable substrate for settling is available. Smith (1935) obtained metamorphosis in few individuals of *P. vulgata*, around 2 to 3 weeks after fertilization. Certain gastropods, as well as the larvae of many other invertebrates, can delay settlement if a habitat suitable for postlarval survival is not available ("delay period," Scheltema 1978).

Our data and the surface water circulation pattern suggest that the gene flow between Porto Moniz and Canico should be mainly

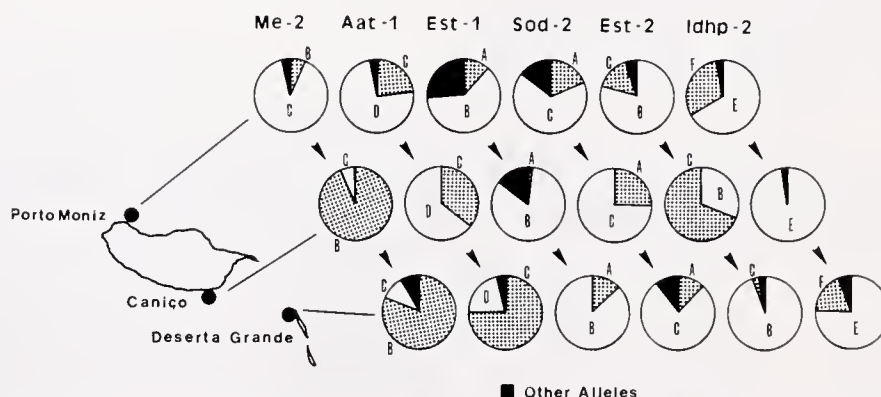


Figure 3. *P. aspera*; geographic patterns of allele frequencies along Madeira Archipelago at the *Me-2*, *Aat-1*, *Est-1*, *Sod-2*, *Est-2*, and *Idhp-2* loci.

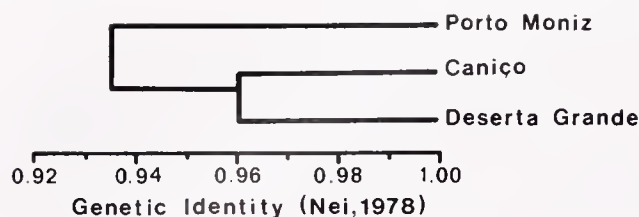


Figure 4. *P. aspera*; UPGMA dendrogram by using Nei's genetic identities between subpopulations.

from the former to the latter around the northern end of Madeira, presumably occurring by genetic interchange of neighboring subpopulations following an isolation by distance model (Slatkin 1993). Deserta Grande should receive migrants through the Canary Current, with major contributions from the south of the Island. Gene flows between Porto Moniz and the north of Deserta Grande may be greater than between Porto Moniz and Caniço, possibly as a result of a more direct contribution of the water-mass movements in the direction of Deserta Grande around the north of Madeira (Fig. 5). An upwelling of triggered water, a consequence of wind stress during summer, could partially prevent the dispersion of larvae through the southern side of Madeira.

Within islands, the structure of subpopulations should follow an isolation by distance model (see Slatkin, 1993); whereas, over a larger scale, between islands, it should follow a stepping-stone model (see Kimura and Weiss 1964). Further analysis using populations from the other archipelagos (Azores and Canaries) is needed to test this hypothesis.

The difference found on the degree of structuring between *P. candei* and *P. aspera* may be explained by one of the following hypotheses. First, *P. aspera* is older than *P. candei*; therefore, it has had more time to diverge intraspecifically and to increase their variability by having more time to incorporate new alleles. Consequently, allowing it even more chances of divergence between localities by either genetic drift or selection. Second, *P. aspera* with a more restrictive habitat (low intertidal to upper subtidal range) has been subject to stronger divergent selection at different localities than *P. candei*, with a broader habitat (from upper intertidal to upper subtidal range). Third, different life histories, could also partly explain this pattern, such as differences in the length of larval life, behavior, and reproductive patterns.

The present work showed us that different biological stocks of the limpets *P. candei* and *P. aspera* are present in Madeira Archipelago. These stocks showed higher variability (for mean number of alleles per locus) than those from the other Macaronesian archipelagos (Weber et al. in prep.). This fact is a further reason to encourage the conservation of Madeiran stocks, because they may

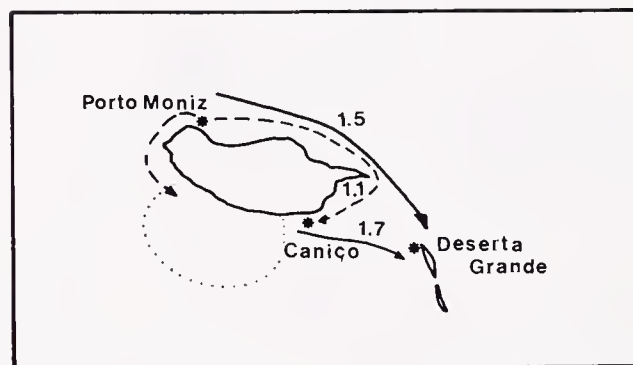


Figure 5. *P. aspera*; probable gene flow patterns at Madeira Archipelago (arrows). Values correspond to number of migrants between localities; (....) summer island mass effect.

constitute sources of variability for other Macaronesian populations.

The difference found in the degree of variability and structuring of populations between *P. candei* and *P. aspera* adds to the list of biological differences that characterize these species. *P. candei* is mainly midtidal; whereas, *P. aspera* is mainly subtidal. Whereas in the Azores, *P. aspera* has a discrete reproductive period, beginning in late summer from August to April, *P. candei* shows spawning for most of the year with only a very short summer resting period (Martins et al. 1987, Menezes, 1991). *P. aspera* is a partial protandrous hermaphrodite (Thompson 1979, Guerra and Gaudencio 1986, Côrte-Real 1992), with males first appearing in the 2nd year, and females appearing during the 3rd year, increasing their proportion to the subsequent year classes. Males are predominant at 13 to 20 mm length size classes and females at 18 to 55 mm length size classes. *P. candei* has no sex change, and individuals reach their maturity between 16 to 20 mm length.

P. aspera subpopulations, with their reproductive mechanism and their restricted gene interchange between them, need special care in their regulation. A "minimum size class" strategy be inadequate for *P. aspera*. Nevertheless, such strategies as that adopted by the regional government from Azores might be adequate for these cases. They imposed laws of rotational openings and closing of grounds. For example, the collection of limpets in the eastern and central group of Azores islands was forbidden during 1989 and 1990, allowing only noncommercial harvest during 1990 in the western groups of islands (see Menezes 1991 and Côrte-Real 1992).

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TABLE 4.

Unbiased genetic identity (above the diagonal) and distance (below the diagonal) between *P. aspera* subpopulations.

Subpopulations	Pto. Moniz	Caniço	Deserta Grande
Pto. Moniz	—	0.930	0.941
Caniço	0.073	—	0.959
Deserta Grande	0.061	0.042	—

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MESOSCALE DISTRIBUTION PATTERNS OF QUEEN CONCH (*STROMBUS GIGAS* LINNE) IN EXUMA SOUND, BAHAMAS: LINKS IN RECRUITMENT FROM LARVAE TO FISHERY YIELDS

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ABSTRACT Populations of benthic species that produce pelagic larvae are sustained through a complex interaction of factors, including larval supply, variable transport mechanisms, and a host of postsettlement processes that affect differential recruitment and abundance. We report distributional data for the larvae, juveniles, adults, and a time-averaged index of fishery yield (shell middens) of the economically important marine gastropod *Strombus gigas* (queen conch) in the Exuma Sound, Bahamas. All life history stages and the fishery yields were heterogeneously distributed around this semienclosed system, with higher densities of benthic stages in the northern part of the sound than in the south and east. Distribution of shell middens closely reflected abundance patterns of shallow-water juvenile aggregations and abundance of adults in depth-stratified surveys; therefore, midden distribution provided a good indicator of long-term productivity around the periphery of the sound. Although patterns of fishery productivity around the system were closely related to both juvenile and adult distributions, and density of newly-hatched larvae reflected the distribution of adults and shell middens, as would be expected, benthic stages and the fishery yields were completely decoupled from the abundance of settlement-stage larvae. When transplants of newly settled conch were made to four seagrass sites in the eastern Exuma Sound with characteristics typical of conch nurseries, low growth rates resulted in all but one location. All of these results suggest that conch abundance and distribution in Exuma Sound is determined in the benthos, either during settlement or in the first year of postsettlement life. Therefore, although larval supply has been shown to influence benthic recruitment on a small scale (i.e., size and location of juvenile aggregations), and juvenile populations will always depend upon a reliable source of competent larvae, high quality habitat plays an equally important role in the recruitment of this important fishery resource.

KEY WORDS: Fishery, habitat, larval supply, mesoscale, oceanography, postsettlement, presettlement, recruitment

INTRODUCTION

Many marine animals have complex life cycles in which they release large quantities of planktonic propagules that are transported by currents to habitats distant from where they were spawned (Doherty and Williams 1988, Roughgarden et al. 1988). Although some marine fishes and invertebrates reduce presettlement losses of larvae by releasing eggs or larvae into transport pathways that favor delivery away from predators and on to appropriate juvenile habitats (Johnson and Hester 1989, Hensley et al. 1994, Morgan and Cristy 1995), the vast majority of these propagules are advected away from suitable settlement habitat, or die during the planktonic or early postsettlement periods. Although all populations that produce pelagic larvae are sustained, to some degree, by the transport of larvae from upstream sources, predation rates on settling and newly settled invertebrates can be very high (Woodin 1976, Osman and Whitlatch 1995, Gosselin and Qian 1997, Stoner et al. 1998), and a host of postsettlement processes can also influence spatial distribution (Hunt and Scheibling 1997). Consequently, the relative importance of pre- and postsettlement processes on distribution has been a subject of much recent research related to invertebrate recruitment (e.g., Olafsson et al. 1994, Eggleston and Armstrong 1995, Wahle and Incze 1997). One way to examine the significance of pre- and postsettlement processes is to test geographic coherence of abundance patterns in all of the life stages, as recommended by Hunt and Schiebling (1997).

The large gastropod mollusk *Strombus gigas* Linne (queen conch) is a convenient model for examining relationships between life history stages for several reasons. First, larvae of *S. gigas* are

readily identifiable at all stages and are relatively large, hatching at about 0.3 mm shell diameter and settling to the benthos at over 1.0 mm in shell length (Davis et al. 1993). Second, juveniles occur in large aggregations, primarily in shallow coastal habitats making them relatively easy to survey. Third, the adults are slow moving, large (to 30 cm), and easily surveyed to their typical depth limit of ~30 m depth. Juvenile and adult conch normally inhabit clear oligotrophic waters, which also facilitates survey work. Fourth, fishers ordinarily land the heavy shells of queen conch on beaches near the collection sites where they extract the edible meat. Because the shells persist on the beaches for at least several hundred years, the shell middens provide an index of historical distribution patterns.

In 1992, the multidisciplinary program FORECAST (Fisheries Oceanography and Recruitment in the Caribbean and Subtropics) was developed at the Caribbean Marine Research Center. The goal of this 5-year program was to provide an understanding of recruitment sufficient to explain mesoscale distribution patterns and interannual variation in economically significant species in Exuma Sound. We have collected distributional data on conch larvae (Stoner and Ray 1996, Stoner et al. 1996a, Stoner and Davis 1997a), juveniles (Stoner et al. 1994, Stoner et al. 1995, Stoner et al. 1996b), adults (Stoner and Schwarte 1994, Stoner and Ray 1996), and shell middens (Stoner 1998) in the Bahamas from as long ago as 1989. In this study, we focus on findings from the FORECAST program related to mesoscale spatial variation in populations of queen conch. We expand our previous analyses of the fishery record and benthic populations to include the entire Exuma Sound system, report new data on synoptic surveys for

queen conch larvae, and investigate patterns of distribution among the interconnected populations of queen conch in this system. We hypothesized that larval production and transport are the dominant factors controlling spatial variation in the distribution of conch larvae, juveniles, and adults in Exuma Sound, and that, as a result, long-term fisheries for conch reflect the general larval supply pattern. We further hypothesized that mesoscale patterns of abundance and distribution may be set during the juvenile stage and mediated by density-dependent postsettlement processes.

Study Site and Background Information

The Exuma Sound is a deep, semienclosed basin located in the central Bahamas that extends for 250 km along an axis, oriented southeast to northwest (Fig. 1). It is bordered to the south, west, and north by the Exuma Cays and the shallow Great Bahama Bank, most of which is sand and seagrass habitat less than 4 m deep. Eleuthera and Cat Island bound the eastern edge of the sound. The only deep-water (>200 m) connection to the Atlantic Ocean is at the southeast end of the sound, between Cat Island and Long Island through a pass that is 50-km wide. The pass between Little San Salvador Island and Eleuthera, 16 km wide, is characterized by a sill with depths to approximately 35 m. West of Cat Island a broad island shelf (to 10-km wide) borders the Exuma Sound. This shelf grades slowly from the intertidal through shallow sand and seagrass habitats to sand and coral near the shelf edge in 15 to 30-m depth. The island shelf along the west coast of Eleuthera and the Exuma Cays is narrow (<1-km wide), grading rapidly from the island shores to 30-m depth. The shelf edge begins at 30 to 35-m depth throughout the sound. Steep slopes descend to depths >2,000 m in the southern part of the basin and to near 1,000 m in the northern part. Because the shelf edge provides a curvilinear boundary between shallow-water conch habitats and the deep Exuma

Sound, distance along the shelf edge was used to standardize abundance patterns for juveniles, adults, and shell middens (see below).

The total human population around the periphery of Exuma Sound is <10,000 people, centered primarily in George Town on Great Exuma. As a result, there are few sources of pollution, fishing pressure on queen conch is relatively low, and the ecology of the system is relatively unspoiled. The semienclosed nature of the sound and the presence of suitable conch habitat make this system a natural laboratory for the study of fishery recruitment processes and for analysis of distribution of conch from larva to adult.

Along the Exuma Cays, juvenile conch live primarily in seagrass meadows on the shallow bank, and adult conch live primarily offshore in the deeper waters of the sound to 30 m (Stoner and Schwarte 1994, Stoner and Ray 1996). Adult conch lay eggs from April through October (Stoner et al. 1992). The prevailing current on the shelf near the Exuma Cays runs alongshore from the southeast to northwest (Colin 1995) and plays an important role in transporting conch larvae to the northwest. Larvae are advected through the numerous tidal passes between the cays and onto the bank (Stoner and Davis 1997a), where competent larvae settle selectively and metamorphose in nursery grounds that have been well studied (Davis and Stoner 1994). Juveniles live in aggregations at densities of 0.1 to 2 individuals/m² (Stoner and Ray 1993, Stoner et al. 1996a). As they mature into adults, juveniles migrate back through the tidal passes and out to the deepwater reproductive areas (Stoner and Ray 1996). Most conch fishers free-dive for their catch from small boats that have limited range. After removing the meat, they discard the shells along the shores of Exuma Sound, thereby creating ever-growing piles near the site of capture. These piles of discarded shells, hereafter referred to as *middens*, provide a time-averaged record of large-scale conch distribution, and a history of the fishery for at least 500 years (Stoner 1998).

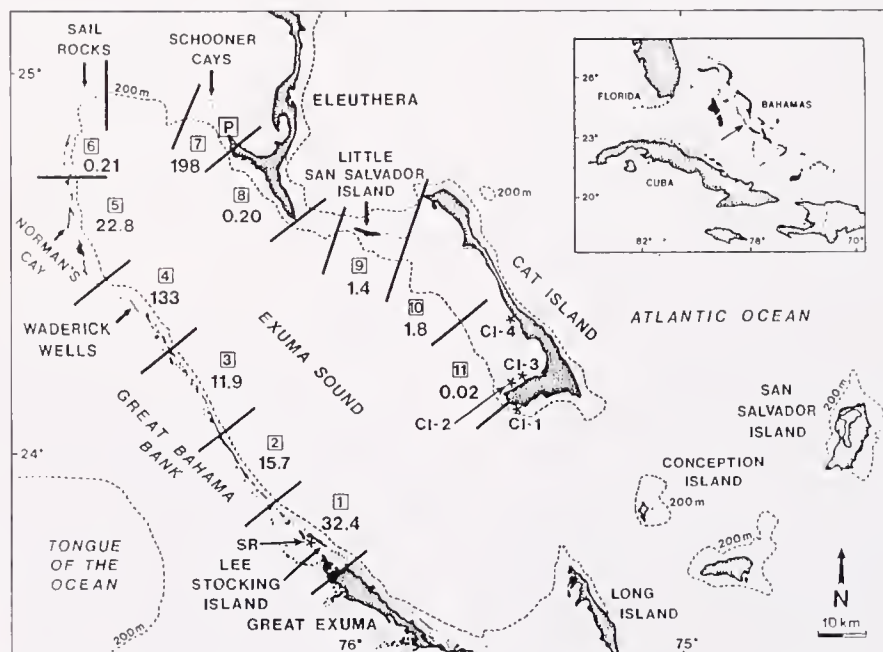


Figure 1. Map of the Exuma Sound system in the central Bahamas. The periphery of the sound was divided into 11 sectors (boxed numbers). The values below the sector numbers indicate the volume of queen conch shell middens expressed in m³ per km of shelf edge (dashed line). Asterisks indicate the five stations at which newly settled conch were transplanted, four at Cat Island (CI-1 to CI-4) and one station at Shark Rock (SR) near Lee Stocking Island. The letter "P" near the island of Eleuthera indicates Powell Point, referred to in the text.

METHODS

Spatial relationships between the abundance of queen conch larvae, juveniles, adults, and discarded shells in middens were examined in and around the Exuma Sound. Surveys for the various life stages spanned several years, and some components of the results, as noted, have been published in previous studies. The methods and results sections will describe the results for different conch stages in reverse ontogenetic order, beginning with middens, because it is this time-integrated spatial record that reflects the long-term fishery that we wish to explain. Furthermore, we were able to quantify shell middens around the entire rim of the Exuma Sound, thereby surveying the entire system. Quantifying all three living conch stages was much more labor intensive, and only regional surveys could be accomplished. A similar survey strategy was used by Lipcius et al. (1997) in an analogous study of spiny lobster (*Panulirus argus*) populations in Exuma Sound.

The purpose of this investigation was not to follow a single cohort of queen conch from larvae to adult stage or to the fishery in the Exuma Sound. Rather, our intent was to examine the long-term record of fishery yields over a relatively large scale (i.e., Exuma Sound) and to interpret it in terms of current abundance patterns observed for early life stages and adults.

Long-Term Record of the Conch Fishery

Shell midden data used in this study were modified from Stoner (1998), where the survey methods were described in detail. Briefly, all of the islands facing the Exuma Sound were searched for shell remains in a clockwise direction from the northern tip of Great Exuma to the southern end of Cat Island between 1989 and 1994 (Fig. 1). The shoreline of Great Exuma was not surveyed, because this island has the largest human population on the periphery of the Sound, and middens have been removed or disturbed by development. Small settlements occur around the rest of the sound, but most of the extensive shoreline is undisturbed.

For this study, the shelf around the sound periphery was divided into sectors that were ~20-km long (Fig. 1). Not included in the division of the periphery were deep-water passes between Cat Island and Long Island, the open-water pass between Little San Salvador and Eleuthera, and the bank periphery where there were no islands for landing conch (i.e., in the extreme northern sound and between Long Island and Great Exuma). Distances were measured at the edge of the shelf and varied somewhat to separate nurseries for queen conch that are associated directly with the tidal flow fields between islands at the edge of the bank (Jones 1996, Stoner et al. 1996b).

Shell middens ranged in size from a few scattered shells to accumulations that were 3 to 4-m high. Estimates of the total volume of individual accumulations were made by measuring their basic dimensions as described by Stoner (1998). Notes were also made on the apparent age of the shell middens. For example, some were composed primarily of very old and eroded shells, and the top layers of others were covered with recently landed shells from which the bright shell nacre had not yet faded. The middens were mapped, volumes were summed for each sector, and the volume of shells per kilometer of shelf periphery was used as a standard index of historic fishery yield from individual bank sectors.

Adult Surveys

Labor-intensive diving surveys for adults were concentrated in four sectors of the sound chosen on the basis of general geographic

positions and known productivity patterns in queen conch revealed in the midden survey. They included: (1) the conch-poor area at the southern end of Cat Island (Fig. 1, sector 11); (2) the well-studied area near Lee Stocking Island in the southern Exuma Cays (sector 1), where conch productivity is moderate; (3) an area inside the Exuma Cays Land and Sea Park near Waderick Wells (sector 4), where Stoner and Ray (1996) found very high densities of adult conch; and (4) an area between the Schooner Cays and the south-east tip of Eleuthera (sector 7), where the highest concentrations of shell middens were located (Stoner 1998).

Stoner (1998) found a positive correlation between middens and juvenile conch abundance, and we hypothesized a similar relationship between middens and adult conch abundance. If such a relationship exists, midden volume could be used as an indicator of living adult conch distribution. Extensive adult surveys were made during the summer 1991 near Lee Stocking Island (Stoner and Schwarte 1994), and near Waderick Wells (Stoner and Ray 1996), Cat Island, and Eleuthera in 1994. Although surveys for conch were made during two different years, the conch populations in the Exuma Sound seem to be relatively stable over the long term. Annual surveys for adult conch conducted at selected sites off Lee Stocking Island between 1988 and 1994 (Stoner and Sandt 1992, unpubl. data) revealed that maximum variation from the mean population size and density was just 19%, and the population was only 4% above average in 1991. This stability is probably a function of low fishing pressure, particularly in depths below the reach of the average free-diving fishers (>10 m), and a queen conch life span of at least 12 years (Coulston et al. 1987).

Depth-stratified surveys for adult conch were conducted in each of the four sectors described above. Seven depth intervals were examined: 0 to 2.5 m (where present), 2.5 to 5 m, 5 to 10 m, 10 to 15 m, 15 to 20 m, 20 to 25 m, and 25 to 30 m. The deepest interval was not surveyed at either Eleuthera or Cat Island because of a very steep grade in depths >25 m that did not seem to support adult conch. The intervals were surveyed along nine offshore transect lines perpendicular to Lee Stocking Island, six lines perpendicular to Waderick Wells, four lines perpendicular to Eleuthera, and three lines perpendicular to Cat Island. Because of extremely low conch densities in the shallow waters (<10 m) near Cat Island, standard swimming transects (described below) were supplemented by extensive observations made by towing one or two divers behind the boat. Densities of approximately zero at most depth intervals (see Results) reflected the results from this more extensive survey method. Total numbers of transects and dives made in each of the four sectors were dependent upon ship time available and logistics. Land-based operations at Lee Stocking Island and Waderick Wells permitted the most intensive surveys.

In each depth interval, two divers swam for 8 to 30 minutes, depending upon depth, holding a taut line (8 m) between them and counting the number of adult conch that lay beneath the line (Stoner and Schwarte 1994, Stoner and Ray 1996). One diver carried a calibrated low-velocity flow meter to calculate the distance traveled. To compensate for the potential influence of current on distance measured, the divers swam into any discernible current and back, covering two parallel, nonoverlapping paths that normally ran parallel to the isobaths. Mean swim distance was 360 m (SD = 106 m), for a typical sampling area of nearly 3 ha. Conch densities were standardized to numbers per hectare. Mean adult conch density was calculated from the replicates at each depth

interval at each sector and then used to generate a final mean representative of that sector for all depths.

Juvenile Surveys

Surveys for juvenile queen conch were conducted in the four sectors of Exuma Sound described above for adults and in sector 5. Juvenile conch in the Exuma Sound region occur in high density (0.1 to 2.0 conch m^{-2}) aggregations and are found almost exclusively in shallow (<5 m-deep) bank habitats (Stoner and Ray 1993, Stoner et al. 1996b). Consequently, aggregations are usually easy to locate in the clear water, but estimations for juvenile abundance require survey techniques different from those used for adults. A detailed description of juvenile mapping technique can be found in Stoner and Ray (1993). In brief, divers were towed systematically over the bank with sufficient intensity to locate aggregations larger than ~1.0 ha in surface area. Once located, the boundaries within which density was greater than ~0.1 conch m^{-2} were determined and marked with buoys. Buoy positions were then determined using hand-held GPS (Global Positioning System). Aggregations were plotted on a small-scale chart, and their surface areas were determined with a calibrated digitizing board. We made exhaustive surveys for juveniles along known lengths of the shelf edge within each of the five sectors, and surface areas of aggregations were standardized per unit of distance along the shelf (i.e., ha of juvenile aggregation/km of shelf).

In the Exuma Cays, juvenile aggregations were located in shallow seagrass meadows to the west of the islands and were directly associated with flood tidal pathways. They were relatively rare in the high energy, windward (east) side of the islands (Stoner et al. 1996b). On the eastern side of the sound, the western shores of the islands are protected from the prevailing tradewind and wave energy, and juvenile aggregations again occur in shallow seagrass meadows immediately to the west of the islands. Searches for juveniles near Eleuthera were concentrated on the shallow bank areas surrounding the Schooner Cays, and on the open, seagrass-covered shelf adjacent to Powell Point. Because aggregations in this area were very large, they were relatively easy to locate and map. Virtually all of the southern bight of Cat Island and 3 km of the southern shore between the shoreline and 5-m depth was searched by towing divers behind small boats in transects separated by no more than 0.75 km. Many days of towing near Cat Island over the entire length of sector 11 (20 km) produced only scattered juvenile conch and no aggregations. All of the surveys were conducted between 1991 and 1993, depending upon the

availability of ship time and other logistics. The general strategy was to search a section at least 10-km long in each of the five selected geographic sectors (see Table 1).

Veliger Surveys

Plankton surveys for conch larvae were conducted between 1993 and 1995, in an attempt to explain the large-scale distribution of queen conch around the periphery of the Sound. The first survey in 1993 comprised simple transects across the Sound in the southwest to northeast direction. In 1994 and 1995, the surveys were made in conjunction with physical oceanographic studies and were expanded for a more synoptic view of the Sound.

Size-specific larval density data are useful tools for interpreting larval production and understanding transport processes. Early-stage, newly hatched queen conch veligers provide an indication of local larval production, and late-stage veligers (2 to 3 weeks old), which may have originated from a distant reproductive population, yield information on the number of conch available to settle into the benthos (Davis et al. 1993; Stoner et al. 1996a).

Queen conch larvae are relatively easy to sample, because they are photopositive (Barile et al. 1994) and most abundant near the sea surface (<5-m depth) when conditions are relatively smooth, as is typical during summer in the Bahamas (Stoner and Davis 1997b). All of the plankton samples collected during the surveys described below were made by towing nets in a stepwise oblique fashion from a depth of 5 m to the surface at ~1 m/sec during daylight hours (except for a subset of 15 stations sampled during the night in 1994, see below). During the first 2 years (1993 and 1994) collections were made with standard conical nets (diam. = 50 cm, mesh size = 0.202 mm) that collect all queen conch larvae including the smallest (~0.3 mm shell length) newly hatched stage. These nets were towed for an average time of 36 minutes, with 12 minutes each at 5 m, at 2.5 m below the surface, and just below the surface. The volume of water sampled, typically 250 to 300 m^3 , was calculated from a calibrated General Oceanics flowmeter suspended in the mouth of the net. Two tows were made at each station.

In 1995, the primary objective was to sample higher volumes of water for late-stage larvae; therefore, net diameter and mesh size were increased to 75 cm and 0.333 mm, respectively. The tow strategy was similar to that used in 1993 and 1994, but total tow time was increased to 45 minutes. Tow volumes with the larger nets were typically 1000 to 1200 m^3 . All plankton samples were preserved in a buffered 5% formalin-seawater mixture.

TABLE 1.

Surface area and concentration of shallow-water juvenile queen conch aggregations in five sectors around the periphery of the Exuma Sound.

Sector No.	General Location	Survey Date	Total Aggregation Area (ha)	Kilometers of Shelf Surveyed	Aggregation Concentration (ha/km)
1	Lee Stocking Island	7/93	129	11	11.7
4	Waderick Wells	2/91	431	17	25.4
5	Norman's Cay	9/91	269	13	20.7
7	Schooner Cays	8/93	650	10	65.0
11	Cat Island	7/93	0	20	0

Juveniles were ≥ 1 year old and were aggregated at densities of 0.1 and 1.0 conch/ m^2 . See Figure 1 for location of each sector.

In 1993, five cruises were made during the peak reproductive season, 12 June to 23 August, on board R/V *Shadow*. During each cruise, a total of 13 stations was sampled along two transects that ran east to west, with one transect across the northern end of Exuma Sound from Waderick Wells to Schooner Cays and the other across the southern sound from Lee Stocking Island to Cat Island (see Results). The four stations at the end of the two transect lines were located in the four sectors that were surveyed for both juvenile and adult conch (sectors 1,4,7,11). The temporal patterns observed during the 1993 plankton surveys (see Results) facilitated the planning of subsequent cruises so that specific larval stages could be targeted, early-stage larvae in the month of June, and late-stage larvae in late August.

In June 1994, two cruises were conducted to provide a synoptic view of veliger density in the Exuma Sound early in the spawning season. On the first cruise (5 to 13 June), 32 stations were sampled from R/V *Sea Diver* along six transects that ran east to west across the sound (see Results). One of the physical oceanographic objectives to be accomplished during this multidisciplinary cruise was the analysis of upper water column circulation. Therefore, 15 stations had to be sampled at night. Although this was not an optimal sampling strategy, Stoner and Davis (1997b) have shown that conch larvae occur in the upper 5 m of the water column during both day and night, under calm conditions. When they occur below 5 m depth, they do so more in response to high wave action and the associated turbulence than to light conditions. On the second cruise (22 to 24 June), 12 additional stations (total of 44) were sampled from R/V *Shadow* at the 20-m isobath along the length of the Exuma Cays (see Results). All samples during this second cruise were collected during daylight hours.

In 1995, two cruises (25 to 31 August and 15 to 17 September) were conducted to obtain a synoptic view of the distribution of late-stage larvae in Exuma Sound during peak settlement period. A total of 41 stations were sampled from R/V *Cyclone* (see Results). The station plan was similar to that employed in 1994, and all collections were made during daytime hours. As mentioned earlier, these collections were made with larger nets and mesh size, to sample late-stage larvae better. Sampling was suspended during a high wind period in early September.

Plankton samples were sorted in their entirety for strombid veligers with the aid of a dissecting microscope. All strombids were identified to species (see Davis et al. 1993, for descriptions), counted, and measured for maximum shell length (SL), but only queen conch veligers (*S. gigas*) are discussed here. The veligers were divided into three general age classes on the basis of size: early-stage (<500 μm SL), midstage (500 to 900 μm SL), and late-stage larvae (>900 μm SL), which were at or near metamorphic competence. Abundance was calculated as numbers of veligers per unit volume of water sampled (veligers/100 m^3) for each age class. Data are reported as the mean of two tows for each station for individual cruises and as mean of means for 1993 when cruises were pooled.

Relationships Among Different Ontogenetic Stages

The abundance and distributional data for middens, adults, juveniles, and larval conch were collected to examine the relationships among distinct ontogenetic stages in different geographic sectors around Exuma Sound. Tests of correlation were performed between conch midden volume and adult conch density, and between midden volume and juvenile conch density. We hypoth-

esized that, if a positive relationship exists between the fishery yield and benthic stages, then midden volume would reflect living adult conch populations and could be used as an index of living conch abundance around Exuma Sound. We also tested the relationship between midden volume and density of early-stage conch veligers along the shelf periphery to determine if newly hatched larvae reflected large-scale distribution of the reproductive stock. To explore the potential importance of larval supply to distribution of benthic populations around the sound, we also tested for correlations between midden volume and density of late-stage larvae and between late-stage larvae and juvenile conch. Individual sectors were often represented by more than one plankton sampling station, providing increased confidence in the values used in the regressions. This varied with year and sampling strategy; however, all plankton stations inside a sector boundary and within 5 km of the shelf edge were included in a mean value.

Transplant Experiment

We observed very low densities of both juvenile and adult conch in the shallow shelf environment at the southern end of Cat Island (see Results). Because late-stage larvae were relatively ubiquitous throughout the sound, it is unlikely that such low density is explained by a low supply of settlement stage larvae to that location. Therefore, we hypothesized that the habitat in this area was unsuitable for juvenile growth and survival. To test this habitat-limitation hypothesis, we conducted a transplant experiment during the summer of 1995 to measure postlarval growth. If the Cat Island habitat was suitable for newly settled conch growth, then postlarvae transplanted there should grow at rates similar to those transplanted in a conch nursery area near Lee Stocking Island called Shark Rock, where a well-studied juvenile aggregation has persisted for over 10 years (Stoner and Waite 1990, Stoner and Ray 1993, Stoner et al. 1994). Although growth rates in enclosures give no indication of predation-induced mortality, they do provide a good index of habitat suitability in terms of food quality and availability (Stoner and Sandt 1991).

Three queen conch egg masses were collected from a reproductive site on the shelf off Lee Stocking Island on 18 June 1995. The eggs hatched 5 days later, and the larvae were cultured according to well-established procedures (Davis 1994). Briefly, larvae were held in 20-L plastic buckets filled with seawater collected daily from the bank west of Lee Stocking Island. Natural foods in the seawater were supplemented with cultured Tahitian *Isochrysis* spp. Metamorphosis was induced on 21 July, at ~1 mm shell length (SL), and postlarvae were raised in plastic trays with aerated seawater on a diet of seagrass detritus (*Thalassia testudinum*) collected from the field.

Five enclosures were deployed at one Shark Rock station in an area of uniform habitat characteristics at a depth of 4.1 m MLW (Fig. 1). It was our intent to deploy the Cat Island enclosures in similar habitat, and, after extensive surveying, four stations were selected along the southwest shore at 3.2–5.3 m depth (Fig. 1). Sediment and seagrass (*Thalassia testudinum*) detritus samples were collected, and living seagrass shoot density was counted near each enclosure to characterize the station (Table 2).

Enclosures were pvc cylinders (diameter = 16 cm, height = 25 cm) with abundant large holes (diameter = 5.5 cm) cut from each to allow for water circulation (after Ray and Stoner 1995). Each cylinder was lined with a polyester mesh (1 mm) sleeve, pushed into the substrata, secured to reinforcement bars driven into

TABLE 2.

Mean growth rate and survival of postlarval queen conch transplanted at four stations near Cat Island (CI) and one station near Lee Stocking Island at Shark Rock (SR).

Station	Growth Rate (mm/day)	Survival (%)	Sediment Type	Seagrass Density (Shoots/m ²)	Seagrass Detritus (g)
CI-1	0.12 ± 0.01 ^a	90 ± 9	Medium sand	570 ± 182	1.35 ± 0.17
CI-2	0.10 ± 0.03 ^a	100 ± 0	Coarse sand	950 ± 178	0.71 ± 0.18
CI-3	0.16 ± 0.01 ^b	90 ± 11	Coarse sand	750 ± 257	0.68 ± 0.28
CI-4	0.30 ± 0.02 ^c	99 ± 2	Medium sand	680 ± 144	0.19 ± 0.13
SR	0.28 ± 0.02 ^c	92 ± 12	Medium sand	710 ± 167	4.51 ± 0.94

Values are mean ± SD; n = 5 at each station except CI-2, where n = 4 for conch growth rate. Growth rate data were homogeneous (Cochran's test, $p > .05$, and differences in the means were determined by one-way ANOVA ($F_{(4,19)} = 114$; $p < .001$) followed by Tukey HSD multiple comparison test. Means that are not significantly different ($p > .05$) are designated by similar lower case letters. Habitat characteristics including sediment type, seagrass (*Thalassia testudinum*) shoot density, and seagrass detrital biomass (dry weight) (n = 5) are also given for each station. See Figure 1 for location of each station.

the sediment, and covered with a mesh top. Large predators were removed prior to introduction of postlarvae.

Prior to transplanting, subsamples of the cultured postlarvae, which were relatively uniform in size, were measured for shell length with dial calipers. Postlarvae were introduced into enclosures (see below) at Shark Rock on 20 August 1995 at 6.0 mm SL (SD = 0.4, n = 40) and at Cat Island on 24 August at 6.1 mm SL (SD = 0.4, n = 80). Each enclosure contained 18 animals.

Postlarvae were recovered from Shark Rock on 9 September, after 20 days in the field, and from Cat Island on 16 September, after 23 days. They were measured for shell length immediately after recovery. Mean daily growth rates were calculated from the living individuals in each cage using the initial shell length from the appropriate subsample. To test for differences among stations, one-way analysis of variance (ANOVA) was performed followed by Tukey HSD multiple comparison test (Day and Quinn 1989).

RESULTS

Long-Term Record of the Conch Fishery

Surveys of shell middens revealed the highly variable nature of the conch fishery yield around Exuma Sound (Fig. 1). Highest shell concentrations (198 m³ shells/km) occurred in the northeast region (sector 7), located between two regions with low concentrations (~0.2 m³/km). A high concentration (133 m³ shells/km) was also observed in sector 4 in the north central Exuma Cays near Waderick Wells. All four sectors in the eastern sound, from Eleuthera to Cat Island, had very low concentrations of conch shells (≤ 2 m³ shells/km), corroborating the low productivities of conch reported by fishers interviewed at Cat Island. Shells were abundant all along the Exuma Cays island chain on the western boundary of the sound, except at the extreme north.

Most of the shell middens in the Exuma Sound contained both very old and recently collected shells. Important exceptions to this were enormous accumulations of recently collected conch in the vicinity of Powell Point on Eleuthera. These shells had bright color indicating capture over the last few years, and most had the thin shell lips indicative of relatively young adults. The largest individual accumulations (>1000 m³) occurred in sector 4 near Waderick Wells, but it was apparent that most of these shells were collected much earlier than those on Eleuthera. This finding was

not unexpected, because sector 4 lies within the Exuma Cays Land and Sea Park, where all fishing has been prohibited since 1985.

Adult Surveys

In general, densities of adult conch were highest at Waderick Wells and Schooner Cays, intermediate near Lee Stocking Island, and very low (except in the 15 to 20-m depth interval) at Cat Island (Fig. 2). Highest density of adults occurred at 10 to 15 m near Waderick Wells (270 conch/ha), at 15 to 20 m near Lee Stocking Island (88 conch/ha), and at 15 to 20 m off Cat Island (84 conch/ha). Distribution at Schooner Cays was bimodal, with density maxima in shallow water (2.5 to 5 m—228 conch/ha) and in relatively deep water (20 to 25 m—93 conch/ha). Most of the conch in the 2.5 to 5-m interval at Eleuthera were very young adults (with thin shell lips), with a high density of large, late-stage juveniles mixed in as noted below. The adults at most other locations and depths were older.

The benthic habitat within the 0 to 2.5-m depth interval comprised very little surface area in each of the four regions surveyed. Adult conch in this narrow band were rare, and, therefore, considered to be negligible. At all four sites, the two depth intervals between 20 and 30 m represented relatively small proportions of the total habitat occupied by adults; therefore, densities of conch in the depth intervals with largest surface area (2.5 to 20 m) were used to test for correlations between adults and other ontogenetic stages (see below).

Juvenile Surveys

Surveys for juvenile aggregations were conducted in five sectors along the periphery of the Exuma Sound (Table 1). The lengths of shelf edge, corresponding to the shallow-water areas surveyed, ranged from 10 km near the Schooner Cays, where conch juveniles were abundant, to 20 km near Cat Island. Scattered juveniles were observed in the bight of Cat Island and along the westernmost third of the south shore, but no aggregations were found during our extensive systematic surveys conducted in 1993 or during numerous visits to the area between 1993 and 1995. Largest aggregations of juvenile conch occurred in the vicinity of the Schooner Cays just north of Powell Point on the island of Eleuthera and on the shelf immediately west of the Point (Fig. 1), where young adults were also abundant. In August 1993, a single aggregation in the seagrass bed extending south from the Schooner

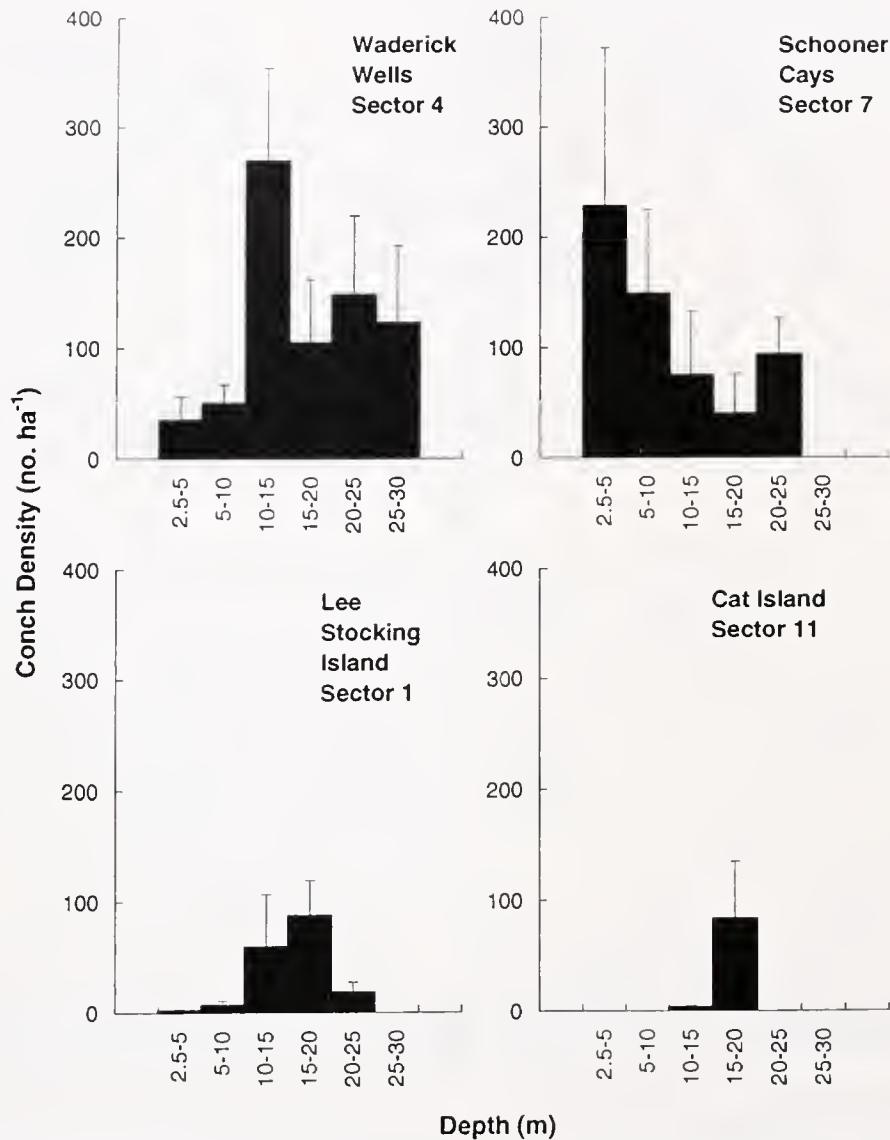


Figure 2. Density of adult queen conch at six depth intervals in the Exuma Sound. Surveys were conducted at four locations, shown here by sector. Values are mean \pm SE. Data for Waderick Wells and Lee Stocking Island are modified from Stoner and Schwarte (1994) and Stoner and Ray (1996).

Cays comprised 610 ha of juvenile conch in densities of at least 0.5 conch/m². Another large aggregation (431 ha) occurred near Waderick Wells. Intermediate concentrations of juvenile aggregations occurred near Norman's Cay (sector 5) in the northern Exuma Cays and near Lee Stocking Island (sector 1) at the southern end of the island chain. Most of the individual aggregations in the Exuma Cays covered between 10 and 100 ha and all were associated with the tidal flow fields immediately west of the inlets and islands in shallow seagrass beds. Repeated observations revealed that the locations of these aggregations were persistent over time.

Veliger Surveys

In 1993, early-stage larvae were most abundant near Waderick Wells (159 to 197 veligers/100 m³), followed by Schooner Cays (29 to 45 veligers/100 m³), Lee Stocking Island (7 to 13 veligers/100 m³), and Cat Island (0 to 5 veligers/100 m³) (Fig. 3A). Early-stage larvae were nearly absent offshore in the open waters of

Exuma Sound. Midstage larvae were concentrated offshore in the northern sound with a mean density of 24 to 41 veligers/100 m³ (Fig. 3B). The mean density of late-stage larvae varied between 0 to 8 veligers/100 m³ at all stations except one offshore in the northern Sound, where mean density was 30 veligers/100 m³ (Fig. 3C). Mid- and late-stage larvae were rarely found along the periphery of the sound during the five surveys conducted in 1993.

As in 1993, early-stage larvae were abundant all along the northern Exuma Cays in 1994, with highest mean densities (208 to 929 veligers/100 m³) near Waderick Wells (Fig. 4A). Stations north of Waderick Wells, near Sail Rocks (see Fig. 1), had mean densities of 65 to 70 veligers/100 m³. The southern part of the sound and the entire eastern periphery yielded low densities of early-stage veligers. For example, Lee Stocking Island, Great Exuma, and Little San Salvador all had intermediate densities of early stages (25 to 59 veligers/100 m³); whereas, Eleuthera and Cat Island had very low densities (0 to 2 veligers/100 m³). As in 1993,

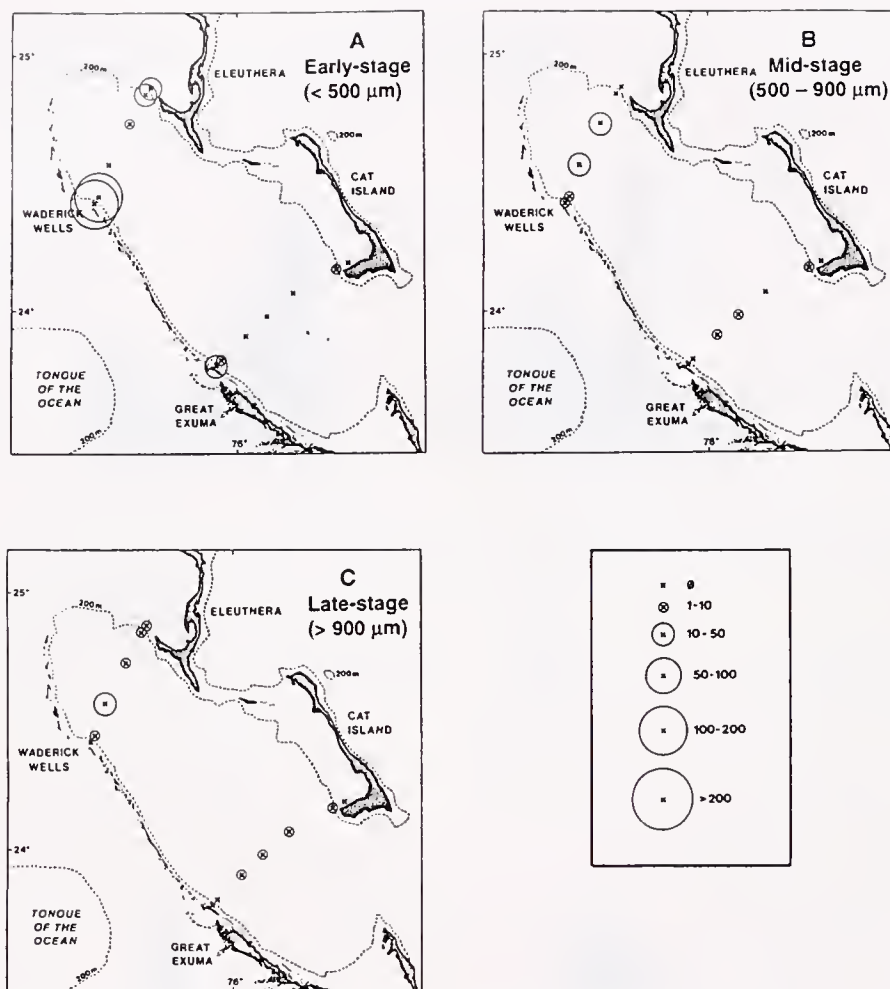


Figure 3. Density of (A) early-stage, (B) midstage, and (C) late-stage queen conch veligers collected during five cruises in 1993 (12 June to 23 August) at 13 stations. Plankton tows were made at each station with 202-µm mesh nets. Values represent the mean of means for each station.

very few early stages were collected at the offshore, open-water stations.

Although mid- and late-stage larvae were widespread throughout Exuma Sound in 1994, they were usually collected in relatively low densities (Figs. 4B,C). Moderate densities of midstage larvae were found along the shelf edge of the northwest sound near Sail Rocks (45 veligers/100 m³), near Waderick Wells (24 veligers/100 m³), and at one station in the center of the Sound (12 veligers/100 m³) (Fig. 4B). The rest of the sound, including its periphery, yielded a mean density of <10 midstage veligers/100 m³. Highest densities of late-stage larvae were found near Sail Rocks (52 veligers/100 m³), the pass between Eleuthera and Little San Salvador (34 veligers/100 m³), the outer shelf edge of Cat Island (56 veligers/100 m³), and one station in the center of the sound (10 veligers/100 m³) (Fig. 4C). The rest of the sound stations yielded 0 to 6 veligers/100 m³. Thus, despite high concentrations of early stage larvae near the large reproductive populations in the north-central Exuma Cays, settlement-stage queen conch larvae were found throughout the sound in relatively low densities.

Veliger distribution was explored along the Exuma Cays in two subsequent cruises, in July and August 1994, and the spatial patterns were remarkably similar to those reported above. For example, highest densities of early-stage larvae were always most

abundant from the middle Exuma Cays to the north, and late-stage larvae were always highest in the extreme northern Exumas.

More intensive surveys for late-stage larvae from late August to mid-September 1995 revealed that these settlement-ready stages were ubiquitous throughout the Exuma Sound, except in the extreme southern sound, in the opening between Cat Island and Long Island, and at numerous stations on the shelf along the Exuma Cays (Fig. 5). Highest densities (10 to 28 veligers/100 m³) occurred in the extreme northern sound, near Waderick Wells, and at a station south of Little San Salvador. The rest of the Exuma Sound had late-stage densities of 1 to 10 veligers/100 m³, with the exception of one station in the central basin (12 veligers/100 m³).

Relationships Among Different Ontogenetic Stages

When the abundance of shell middens in a sector was compared with the mean density of adult conch on the adjacent shelf <20 m in depth (Sectors 1, 4, 7, and 11), there was a highly significant correlation ($r = 0.973$, $p = .03$) (Fig. 6A). Midden abundance was also closely correlated with the abundance of juveniles in adjacent waters (Sectors 1, 4, 5, 7, and 11) ($r = 0.915$, $p = .03$) (Fig. 6B). The correlations between shell midden volumes and living populations of both juvenile and adult conch

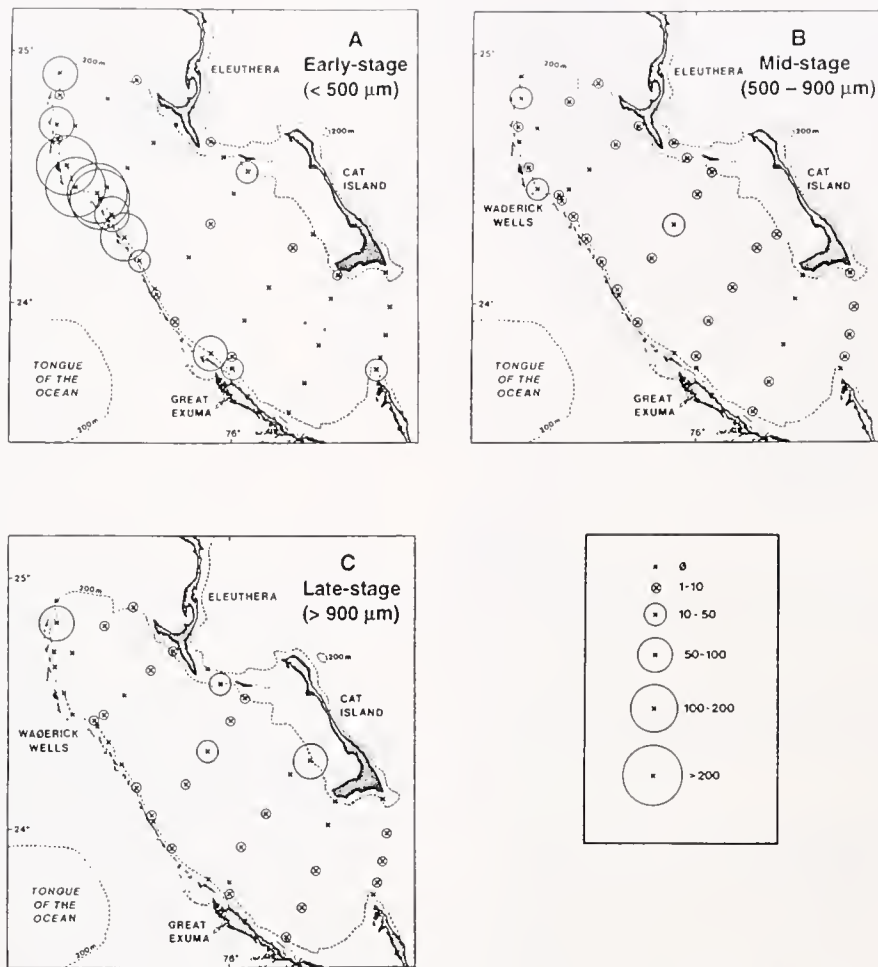


Figure 4. Density of (A) early-stage, (B) midstage, and (C) late-stage queen conch veligers collected during two cruises in 1994 (5 to 13 June, and 22 to 24 June) at 44 stations. Plankton tows were made at each station with 202- μ m mesh nets. Values represent the mean for each station.

over the mesoscale validates the use of middens as a proxy indicator of living conch abundance around the perimeter of Exuma Sound.

Abundance of early-stage, newly hatched larvae at any one location should reflect the size and/or density of the reproductive population in the general vicinity. The most synoptic data for early stages were collected in 1994 (Fig. 4), and there were 10 sectors for which we had both veliger and midden data. Very high concentrations of larvae were collected in the north-central Exuma Cays and near Lee Stocking Island in the southern Exumas. Unexpectedly, the correlation between early-stage larval densities and midden abundance was low and not significant ($r = 0.413$, $p = .24$). The poor correlation was a function of one extreme outlier representing sector 7, near the Schooner Cays, where very large populations of adult conch and large middens were found, but few early-stage veligers. As mentioned above, most of the adult conch at this site were very young adults, which may not have been in reproductive state in the summer of 1994. Also, unlike other in-shore shelf stations around the sound, the stations that we sampled for veligers near the Schooner Cays were swept by very strong tidal currents; therefore, it is possible that sampling at this site during the flood tide resulted in low larval densities. The flood tide would carry locally spawned larvae onto the adjacent bank and away from the sampling stations. When sector 7 was removed

from the analysis, there was a highly significant positive correlation between the abundance of early larval stages and middens ($r = 0.964$, $p < .001$), as was predicted. Best distribution of residuals occurred with a natural log transformation of the data ($r = 0.746$, $p = .02$) shown in Figure 7.

We also hypothesized that the juvenile abundance pattern (Table 1) would reflect densities of late-stage larvae (i.e., those that are at or near metamorphic competence and ready to settle). However, using the juvenile abundance data available for five sectors (Table 1), the correlations were low and not significant ($p > .35$) in all 3 years in which larval data were collected ($r = 0.431$ in 1993, $r = 0.512$ in 1994, $r = 0.220$ in 1995) (Fig. 8). In 1994, high densities of late-stage larvae were found at the south end of Cat Island (sector 11) (Fig. 4), where juvenile populations were typically very small. In the same year, sectors with large juvenile populations (e.g., sectors 4 and 5) had low densities of late-stage larvae. In 1995, late-stage larvae were relatively high in sectors 4 and 5, but also common in sector 11 near Cat Island (Fig. 5). Larval supply was not a good predictor of juvenile concentration.

To complete the analysis of the relationship between middens and mesoscale distribution of conch around the Exuma Sound, we examined midden volume as a function of late-stage, competent larvae. The correlations were negative and not significant in 1994 ($r = 0.420$, $p = .23$, $n = 10$) and in 1995 ($r = 0.312$, $p = .35$,

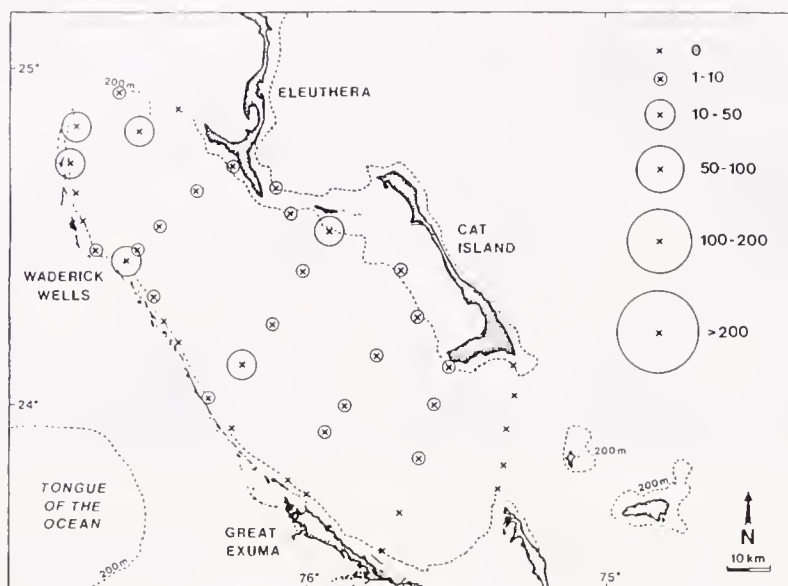


Figure 5. Density of late stage queen conch veligers collected during two cruises in 1995 (25 to 31 August and 15 to 17 September) at 41 stations. Plankton tows were made at each station with 333- μ m mesh nets. Values represent the mean for each station.

$n = 11$) (Fig. 9). As with juvenile distribution, larval supply was not a good predictor for the distribution of fishery yields.

Transplant Experiment

Most (90 to 100%) of the conch transplanted at Cat Island and in the Shark Rock conch nursery were recovered from their enclosures alive, except for one cage at station CI-2, where all conch were lost for unknown reasons (Table 2). Growth rates were significantly higher (0.3 mm/day) at both CI-4 and Shark Rock than at the three other Cat Island stations (0.1 to 0.2 mm/day) (Table 2). Growth was independent of seagrass shoot density and seagrass detritus.

DISCUSSION

It is widely recognized that fishing is better at some locations than others and that this variation occurs over both small and large scales. Variation in the abundance of exploited benthic animals that have pelagic larvae can be explained by differences in: (1) larval supply; (2) larval settlement; (3) the amount and quality of habitat for juveniles; (4) survival to the size at which the animals enter the fishery; and (5) fishing mortality. It is clear that long-term landings of queen conch are not homogeneously distributed around Exuma Sound (Stoner 1998, this study). The purpose of this investigation and the following discussion is to examine the meso-scale patterns of abundance and distribution of all queen conch stages and to determine the point in the life history at which the observed patterns of fishery landings are set.

Radiocarbon dates for shells in middens along the periphery of the Exuma Sound show that these middens provide a historical record of the queen conch fishery spanning several hundred years (Stoner 1998). Although the pattern of conch exploitation is independent of the distribution of human settlements around the sound, there were high correlations between the volume of shell middens and the abundance of both living adults ($r = 0.97$) and juveniles ($r = 0.92$). Thus, midden volumes provide a long-term record of fishing productivity around Exuma Sound as well as an indirect index of living conch distribution. The close correlations between

cumulative landings and local conch populations suggest that the mechanism of distribution occurs somewhere in the life history of conch prior to the age-1 and age-2 year classes that were quantified in the juvenile surveys.

Spatial variation in the adult conch populations was reflected in

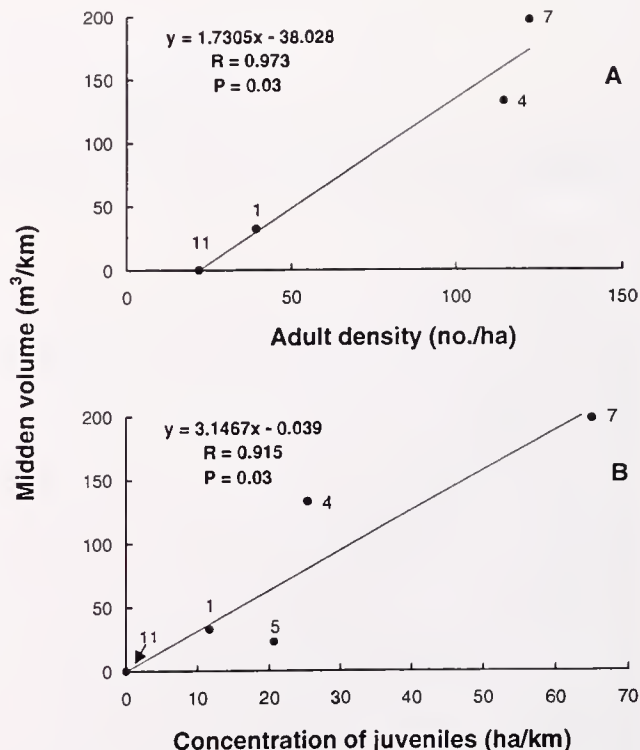


Figure 6. Conch midden volume plotted as a linear function of (A) adult conch density at 2.5 to 20-m depth and (B) concentration of juvenile conch. Pearson correlation coefficients (R) and p -values are given for each regression equation. The number above each point represents the sector at which surveys were conducted in Exuma Sound.

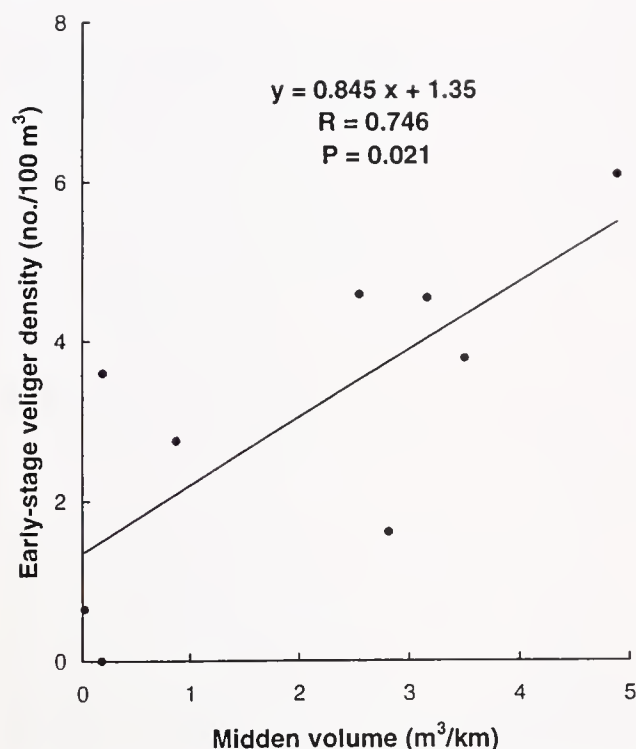


Figure 7. Relationship between mean density of early-stage (<500 µm) conch veligers (natural log transformed) and conch midden volume, surveyed at nine sectors in Exuma Sound in 1994. The Pearson correlation coefficient (R) and p-value are given for the regression equation.

the production of early-stage larvae around the periphery of Exuma Sound. These newly hatched larvae were most abundant in the nearshore areas where adults live and in regions known for high adult concentrations, such as near the Schooner Cays and in the Exuma Cays Land and Sea Park near Waderick Wells. The positive correlation between adults (i.e., spawner abundance) and early-stage larvae was predictable and not surprising.

Ultimately, however, it is settlement-stage larvae that supply and sustain benthic populations, and examples of correlations between larval supply and settlement and/or recruitment to various benthic stages are known for a variety of marine invertebrates (Caffey 1985, Keough, 1988, Bertness et al. 1992) and fishes (Milicich et al. 1992, Doherty and Fowler 1994). Relationships between late-stage larval concentration and juvenile population size have been explored for queen conch in several different locations and on different scales. Stoner and Davis (1997a) have shown that aggregations of juvenile conch were directly associated with local concentrations of late-stage larvae within a tidal current flow field (<10-km long) on the Great Bahama Bank near Lee Stocking Island. Significant positive correlations have also been found between densities of late-stage larvae and juvenile population size on a 10 to 50-km scale across multiple nursery grounds in the Exuma Cays and in the Florida Keys, although the pattern was not coherent across the two locations (~500 km) (Stoner et al. 1996a).

Because of the semiencloded circulation pattern in the Exuma Sound (Colin 1995, Lipcius et al. 1997), it is probable that conch larvae produced in the sound could be retained in the system for the duration of their developmental period. However, the potential

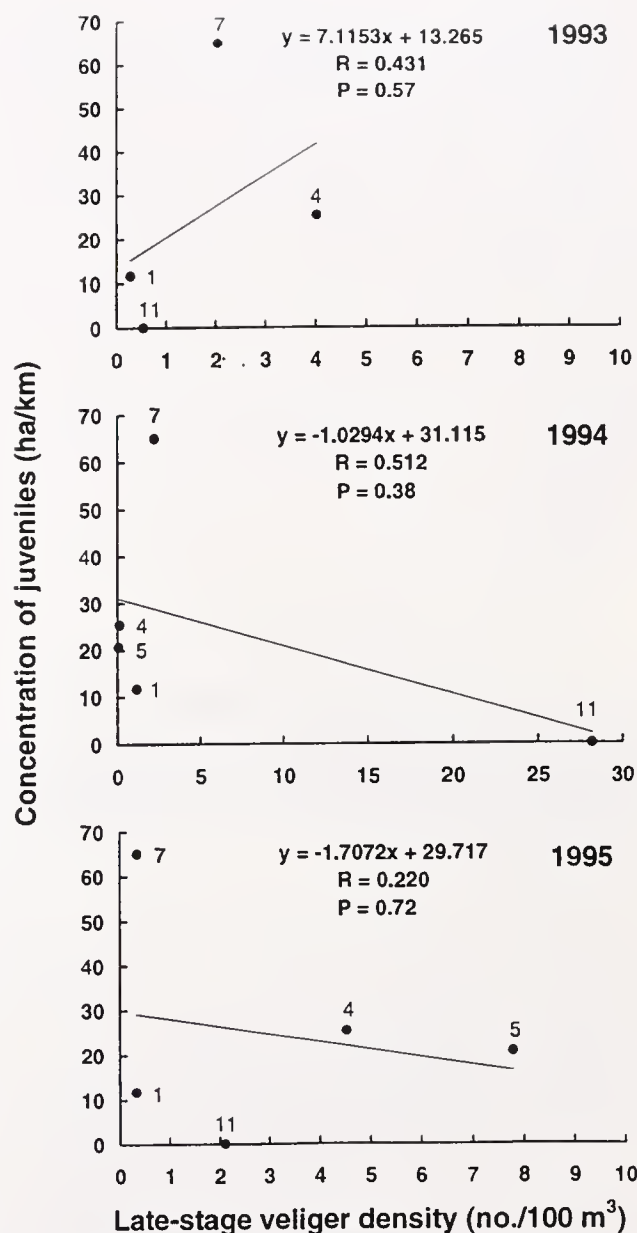


Figure 8. Linear relationship between the concentration of juvenile conch and mean density of late-stage (>900 µm) conch veligers from 1993 to 1995. Pearson correlation coefficients (R) and p-values are given for each regression equation. The number above each point represents the sector at which the survey was conducted in Exuma Sound. Note extended x-axis for 1994 data.

for dispersion within the sound over the 2 to 4 week precompetent period (Davis 1998) is very large. Although late-stage conch larvae were consistently abundant in the northern Exuma Sound during 1994 and 1995, they were relatively ubiquitous throughout the sound. Correlations between the concentrations of settlement-stage larvae and either juvenile or midden distributions were never significant in any of the 3 survey years despite consistent spatial patterns of larval density. These results suggest that the regional pattern of distribution in benthic life stages within Exuma Sound is set by settlement processes and/or early postsettlement processes during the first year of life, and not by differences in larval supply. Lipcius et al. (1997) arrived at similar conclusions about the large-

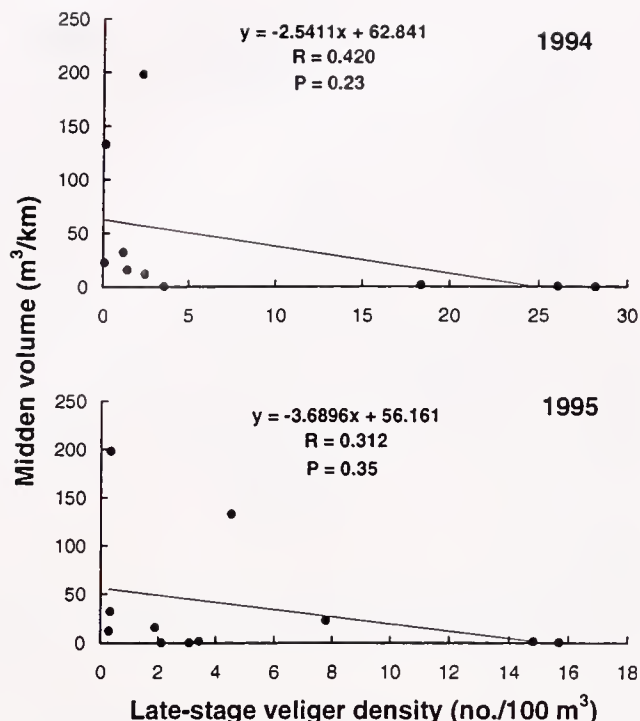


Figure 9. Linear relationship between queen conch midden volume and mean density of late-stage ($>900 \mu\text{m}$) conch veligers in 1994 and 1995. Pearson correlation coefficients (R) and p-values are given for each regression equation. Symbols represent sectors surveyed in Exuma Sound for the 2 years.

scale distribution of spiny lobster (*Panulirus argus*) populations in Exuma Sound. Settlement stage lobster were abundant at Cat Island, yet benthic populations were small. The decoupling between larval supply and juvenile and adult stages was attributed to habitat limitation for early juvenile lobster at Cat Island.

Miron et al. (1995) pointed out the inherent methodological difficulties in correlating larval supply and larval settlement or recruitment. They noted that competent larvae must be quantified and that they must be sampled properly (i.e., with proper respect to location in the water column and settlement substratum). Although settlement-stage queen conch larvae have never been collected in high densities, compared with densities of other mollusks in temperate waters, they are relatively easy to sample, because they occupy near-surface waters in most circumstances (Barile et al. 1994, Stoner and Davis 1997a, Noyes 1996). Furthermore, it is relatively easy to identify competent forms on the basis of size, pigmentation, and other features (Davis 1998). Consequently, we believe that we have sampled the correct larval stages using an appropriate technique.

It can also be argued that larval supply is best measured as a rate of delivery of competent larvae to a potential settlement site (Olmi et al. 1990, Yund et al. 1991). Measuring this is particularly difficult for queen conch on the Great Bahama Bank because of strong tidal currents (see Stoner and Davis 1997b). However, the difficulty is lower in the Exuma Sound, and we have high confidence in the regional patterns of larval abundance reported for two reasons. First, currents in the sound are much weaker ($<20 \text{ cm/sec}$; Colin 1995), than those on the Bank (often $>100 \text{ cm/sec}$; N. P. Smith, unpubl. data), so the issue of larval flux is less complicated in the sound than on the nursery grounds of the Bank. Second, and

more importantly, multiple visits to selected stations throughout the sound between 1993 and 1994 revealed that the regional patterns in veliger distribution were consistent over time. For example, five cruises over 13 stations in 1993 showed that early- and midstage larvae were always abundant in the northern sound, and always highest on the shelf adjacent to Waderick Wells. Late-stage larvae were always most abundant in the sound offshore from Waderick Wells. Three cruises along the island shelf east of the Exuma Cays in 1994 (Stoner and Mehta, unpubl. data) confirmed the pattern of maximum abundance of early- and midstage larvae in the vicinity of Waderick Wells and to the north, and late-stages were most abundant near Sail Rocks in every case. Larvae of all stages were always rare in the extreme south section of the sound. Therefore, because of the consistency of larval distribution, both within and between years, we believe that the regional patterns of larval abundance reported in this study are representative for the sound.

Given that the abundance of late-stage larvae did not explain mesoscale variation in the abundance of juveniles, adults, or fishery yields of queen conch in the Exuma Sound, we conclude that the regional distribution of benthic stages is regulated by settlement and/or postsettlement processes associated with some element of the habitat. Similar conclusions have been drawn for a large number of other marine invertebrates (Keough and Downes 1982, Luckenbach 1984, Connell 1985, McGuinness and Davis 1989, Osman et al. 1992, Olafsson et al. 1994, Eggleston and Armstrong 1995, Hunt and Scheibling 1997, Lipcius et al. 1997).

Many invertebrates settle and metamorphose in the presence of certain chemical agents in or on the substratum (Morse and Morse 1984, Hadfield and Scheuer 1985, Burke 1986, Butman and Grassle 1992, Pawlik 1992), and Mianmanus (1988) has shown that phycobiliproteins associated with red algae are active agents in conch settlement and metamorphosis. We know from extensive dredge sampling for newly settled queen conch (both live and recently killed) in a tidal flow field near Lee Stocking Island that settlement is not random and that it occurs in specific locations (Stoner et al. 1998). This confirms earlier laboratory experiments showing that competent queen conch larvae settle in response to specific biological cues found within nursery grounds (Davis and Stoner 1994). Queen conch larvae are, in fact, capable of testing the substratum, returning to the water column multiple times, and delaying metamorphosis for long periods of time (for at least 60 days after competence is achieved) (Noyes 1996). Experimental laboratory work shows that the larvae settle and metamorphose only in habitats where subsequent growth rates are high (Stoner et al. 1996c). Consequently, it is possible that variation in the abundance of queen conch populations on the Great Bahama Bank surrounding the Exuma Sound is related to either the quality or quantity of habitat with appropriate settlement cues and high growth potential for postlarvae.

Habitat-limitation is the most plausible explanation for the low abundance of juvenile and adult conch west of Cat Island, because competent larvae were present in substantial numbers. Frequency of settlement was not tested, because the only way to ascertain this is by dredging, which is extremely labor intensive. However, transplant experiments provide important insights into the nutritional quality of potential nursery sites. Two lines of reasoning suggest that habitat at Cat Island is limiting for conch. First, the type of habitat that typically supports juvenile conch on the Great Bahama Bank (moderate density seagrass with accumulations of decomposing detritus and red and green algae) has been studied exten-

sively (see Stoner 1997), and was uncommon on the bank west of Cat Island. Seagrass was found in relatively small patches (1 to 10 ha), and much of this was exposed to higher physical energy than is typical for conch nurseries. Second, only one of the four sites assumed to be suitable for juvenile conch provided for growth rates similar to those in a known nursery near Lee Stocking Island. Therefore, it is likely that the small queen conch population and the poor fishery for conch near Cat Island is habitat-limited.

Differential mortality of young conch could also explain regional variation in recruitment to the age-1 year class. There are a host of predators on juvenile conch (Randall 1964), including a large variety of recently discovered micropredators such as xanthid crabs and certain polychaetes that feed on newly settled conch (Ray-Culp et al. 1997). It is now recognized that mortality rates in newly settled invertebrates can be very high (Osman and Whitlatch 1995, Gosselin and Qian 1997), and queen conch are no exception (Ray et al. 1994, Stoner and Glazer 1998). Although we did not test for regional variation in mortality of juvenile conch, this is a possible explanation for the population patterns observed.

Conclusions and Fishery Management Implications

Genetic analysis of queen conch collected from 22 populations throughout the greater Caribbean region, including the Bahamas and south Florida indicate a high rate of gene flow among the populations (Mitton et al. 1989, Campton et al. 1992), and certain populations may depend entirely upon upstream reproductive sources (Stoner et al. 1997c). It is clear, therefore, that sound fisheries management will demand good knowledge of larval drift and associated metapopulation dynamics (Berg and Olsen 1989, Appeldoorn 1994, Stoner 1997). However, the direct correlation between the quantity of larvae supplied to the nurseries and the

subsequent abundance of juvenile queen conch in the benthic population that occurs at a local scale (Stoner et al. 1996a) seems to break down at the large scale. In Exuma Sound (180-km long) the abundance of early-stage larvae was positively correlated with regional abundance of adults. However, the distribution of juveniles, adults, and fishery yields was independent of the abundance of competent larvae, and processes of settlement and postsettlement seem to regulate benthic population size. We have shown in the past that conch nursery grounds have unique physical and biological features that enhance larval settlement, provide high nutritional qualities, and promote high survivorship (Stoner et al. 1995, Stoner 1997). It is now clear that high abundance of competent larvae does not guarantee high queen conch production, and that fisheries management for the species must consider both qualitative and quantitative elements of habitat for young conch. Because vast shallow-water areas within the biogeographic range of queen conch are, in fact, not suitable for production of the species, both local- and large-scale mechanisms of population dynamics and habitat use need to be understood, and the ecological integrity of key nursery habitats needs to be preserved.

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ALLOZYME AND MORPHOLOGICAL EVIDENCE SUPPORTING THE SEPARATION OF *BABYLONIA FORMOSAE FORMOSAE* FROM *B. FORMOSAE HABEI* AT SPECIFIC LEVEL (PROSOBRANCHIA: BUCCINIDAE)

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ABSTRACT *Babylonia formosae* is a common species on the west coast of Taiwan. From its shell color pattern and shape, two subspecies have been classified: *B. formosae formosae* (Sowerby 1866) and *B. formosae habei* (Altena and Gittenberger 1981). Recently, both subspecies were collected from the same area, which was not in accordance with previous recorded harvests. Therefore, the present study was undertaken to evaluate the validity of the subspecies. *B. areolata* occurring in the same area was also examined for comparative purpose. Samples of *B. formosae formosae*, *B. formosae habei*, and *B. areolata* were collected between September 1990 and March 1991. Six shell characters (shell length, shell width, spire length, apertural length, fasciole ridge width, and shoulder width), radulae, and allozymes were analyzed. The shoulder width of shell could separate species with *B. formosae habei* > *B. areolata* > *B. formosae formosae*. Fixed allelic differences were observed at loci of *ark*, *got-1*, *mpi*, and *pgm* between *B. formosae* and *B. areolata*, and at loci of *ark*, *got-1*, and *mpi* between *B. formosae formosae* and *B. formosae habei*. Nei's genetic distances (D) were 0.25 for *B. formosae formosae* vs. *B. formosae habei*, 0.35 for *B. formosae formosae* vs. *B. areolata*, and 0.37 for *B. formosae habei* vs. *B. areolata*. The mean heterozygosity among populations were low in *B. areolata* ($H_o = 0$ to 0.06), *B. formosae formosae* ($H_o = 0.07 - 0.08$), and *B. formosae habei* ($H_o = 0.06$ to 0.07). All the above results indicated that the two subspecies deserve to be recognized as full species: *B. formosae* and *B. habei*.

KEY WORDS: *Babylonia*, ivory snail, allozyme, shell, radula

INTRODUCTION

Babylonia formosae (Sowerby 1866) belongs to the family of Buccinidae. Its distribution was limited to the west coast of Taiwan (Altena and Gittenberger 1981). Within this small region, two subspecies have been classified: *B. formosae formosae* (Sowerby 1866) and *B. formosae habei* (Altena and Gittenberger 1981) (see Table 1). According to Altena and Gittenberger (1981), *B. formosae habei* was found only in the northeast coast of Taiwan. However, our investigation in late 1990 revealed that *B. formosae formosae* and *B. formosae habei* both were commonly caught on the southwest coast of Taiwan. Moreover, Lan (1990) mentioned that *B. formosae habei* sold in Taiwan may be imported from China. Ke and Li (1991) and (Ke and Li 1992) also reported that *B. formosae habei* is a commercially important shellfish on the southeast coast of China. Meanwhile, studies of reproduction found that *B. formosae habei* spawns between June to September (Ke and Li

1991) (Ke and Li 1992); whereas, *B. formosae formosae* spawns between October to January (Chiu and Liu 1994). Although the two subspecies have been studied since 1991 by Ke and Li (1991) (Ke and Li 1992) and Chiu & Liu (1994), it is still difficult to evaluate the systematic status of the two subspecies at this moment. Hence, the present paper studied the systematic status of the two subspecies using morphological, radula, and allozyme characters. *B. areolata* (Link 1807) was also studied for comparative purpose. *B. areolata* is the most common *Babylonia* species in Taiwan. Its distribution is from Ceylon and the Nicobar Islands through the Gulf of Siam, along the Vietnamese and Chinese coasts to Taiwan (Altena and Gittenberger 1981).

Both *B. formosae* and *B. areolata* live in sandy or muddy subtidal areas and can be caught by either bottom trawling or in baited baskets at depths of 15 to 50 m (Lai 1987).

MATERIALS AND METHODS

Samples of *B. areolata*, *B. formosae formosae*, and *B. formosae habei* were collected from coastal waters of Taiwan (Fig. 1) between September 1990 and March 1991. Collected snails were stored at -70°C for later use. Voucher specimens were deposited in the Molluscan Collection, University of Colorado Museum (UCM), with the catalog numbers UCM 37665 for *B. areolata*, 37666 for *B. formosae formosae*, and 37667 for *B. formosae habei*.

Species were identified preliminarily using the color-pattern criteria (Altena and Gittenberger 1981). Species of *B. areolata* has three rows of large reddish brown squarish spots on the last body whorl (Fig. 2a,b). *B. formosae* has four rows of violet-brown spots on the last body whorl. The spots clearly contrast with the light background, and the color is brighter in *B. formosae formosae* (Fig. 2c,d) than in *B. formosae habei* (Fig. 2e,f).

Shell characters (shell length, shell width, spire length, apertural length, fasciole ridge width, and shoulder width) (Fig. 3) and total wet weight of individual snails were determined. Duncan's

TABLE 1.

Diagnostic Subspecies Characters of *Babylonia formosae* Based on Altena and Gittenberger (1981).

Diagnostic Characteristics	<i>B. formosae formosae</i>	<i>B. formosae habei</i>
Last body whorl	Evenly rounded	Less evenly rounded
Shoulder on the last body whorl	Narrower	Narrow
Suture on the last body whorl	Narrow	Narrower
Spots on the last body whorl	Distinct	Less distinct
Color pattern of the spots	Bright	Dull
Distribution	Northwest to southwest coast of Taiwan	Northeast coast of Taiwan

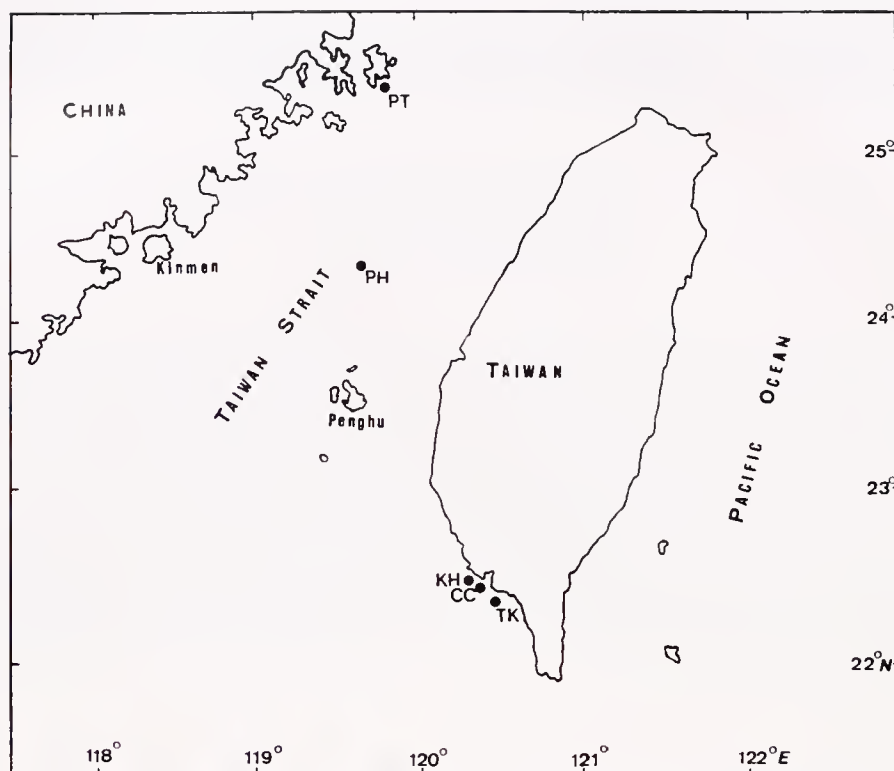


Figure 1. Sampling localities of *Babylonia*. KH: Kaohsiung; TK: Tungkang; PH: Penghu; CC: Chungchou; PT: Pyngtan.

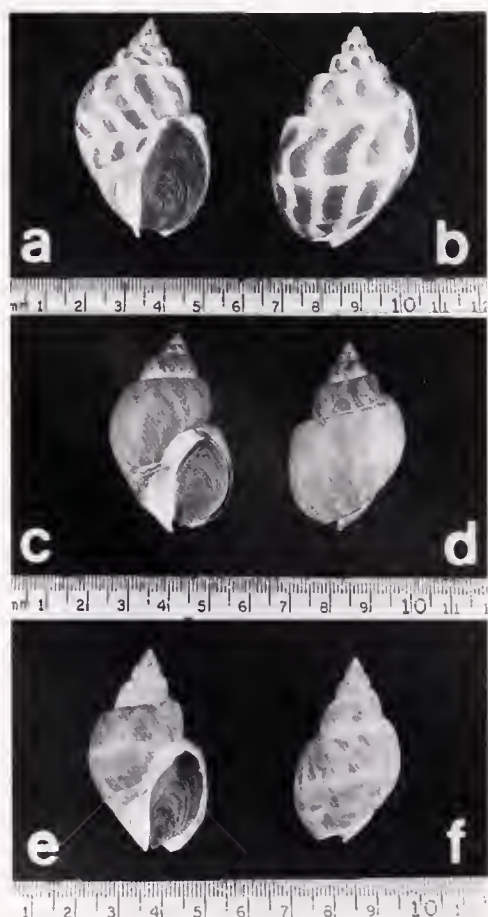


Figure 2. Shells of *Babylonia areolata* (a, b), *B. formosae formosae* (c, d), and *B. formosae habei* (e, f).

multiple comparison tests were used for data analysis (SAS Institute, Inc. 1985).

For radula examination, six buccal masses of each species were put in a 10% KOH solution overnight to resolve muscle and connective tissue around the radula. Each radula was then rinsed in distilled water and ultrasonically cleaned for 30 seconds. The

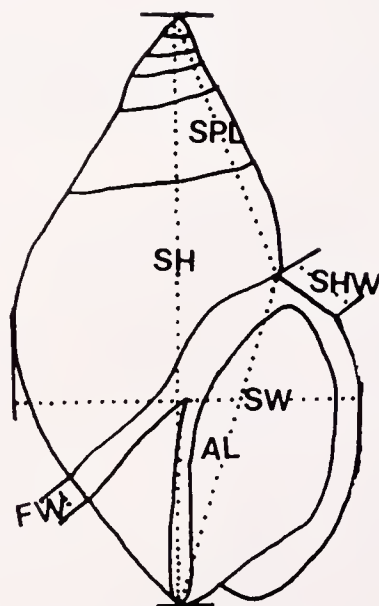


Figure 3. Measurements of the shell of *Babylonia*. SH: shell height; SW: shell width; SPL: spire length; AL: apertural length; SHW: shoulder width; FW: fasciole ridge width.

TABLE 2.
Measurements of Shell Characteristics of *Babylonia* Species (Mean \pm SD).

Species/ Sample Location	n	Shell Length (mm)	Shell Width (mm)	Spire Length (mm)	Apertural Length (mm)	Fasciole Ridge Width (mm)	Shoulder Width (mm)	Total Weight (g)
<i>Babylonia areolata</i>								
KH	30	54.7 \pm 3.3 B	33.0 \pm 1.4 B	29.0 \pm 2.2 B	32.6 \pm 1.2 B	4.7 \pm 0.4 A	3.0 \pm 0.5 C	29.2 \pm 3.7 B
TK	28	55.3 \pm 16.2 B	32.2 \pm 8.6 B	29.7 \pm 9.6 B	30.6 \pm 8.1 C	4.4 \pm 1.2 B	3.3 \pm 1.1 C	31.7 \pm 19.3 B
PH	30	42.9 \pm 8.5 C	26.8 \pm 4.9 C	21.9 \pm 5.3 D	26.5 \pm 4.6 D	3.8 \pm 0.8 C	3.0 \pm 0.6 C	17.7 \pm 9.6 C
PT	28	65.2 \pm 7.7 A	38.2 \pm 3.6 A	35.3 \pm 4.8 A	37.1 \pm 3.4 A	5.0 \pm 0.7 A	3.7 \pm 0.6 B	46.3 \pm 11.9 A
<i>B. formosae formosae</i>								
KH	55	45.0 \pm 6.0 C	26.1 \pm 2.9 CD	24.9 \pm 3.7 C	24.7 \pm 2.9 E	3.6 \pm 0.5 CD	2.3 \pm 0.5 D	15.9 \pm 5.5 CD
TK	50	41.6 \pm 6.2 CD	24.6 \pm 3.3 D	22.3 \pm 4.0 D	23.2 \pm 3.0 EF	3.3 \pm 0.6 E	2.0 \pm 0.5 E	13.0 \pm 5.1 DE
<i>B. formosae habei</i>								
CC	66	43.0 \pm 5.3 C	25.5 \pm 3.2 CD	22.2 \pm 3.2 D	24.5 \pm 3.0 E	3.9 \pm 0.6 C	4.3 \pm 0.8 A	14.4 \pm 5.7 CDE
PT	30	38.7 \pm 4.1 D	22.3 \pm 2.5 E	20.0 \pm 2.4 D	22.1 \pm 2.3 F	3.4 \pm 0.6 DE	4.1 \pm 0.5 A	10.2 \pm 3.5 E

n: sample size. Duncan's test for significant variation is indicated by capital letters ($p < .05$).

cleaned radulae were dried and mounted on SEM stubs. These specimen were then gold-coated and examined with a Hitachi 450 scanning electron microscope at 15 KV.

For allozyme studies, foot tissue (0.2 to 0.5 g) was taken and homogenized in a Tekmar tissumizer with an equal volume of 10 mM Tris-HCl buffer (pH 7.0) containing 1% Triton X-100. Homogenates were centrifuged at 5,000 g for 10 min, and the supernates were stored at -70°C . Horizontal starch-gel electrophoresis with buffer systems Tris-citrate pH 8.0, Tris-citrate pH 6.3/6.7, Tris-maleate-EDTA pH 7.4, and lithium hydroxide pH 8.1/8.3 was used. Enzyme-staining methods followed Richardson et al. (1986) and Murphy et al. (1990).

Multiple loci encoding the same enzyme (isozymes) were designated by consecutive numbers, with 1 denoting the slowest migrating isozyme. Twelve enzyme loci were scored: arginine kinase (*ark*, EC 2.7.3.3); esterase (*est*-1,2, EC 3.1.1.1); glutamate-oxaloacetate transaminase (*got*-1,2, EC 2.6.1.1); isocitrate dehy-

drogenase (*idh*, EC 1.1.1.42); malate dehydrogenase (*mdh*, EC 1.1.1.37); mannose-6-phosphate isomerase (*mpi*, EC 5.3.1.8); octopine dehydrogenase (*opdh*, EC 1.5.1.11); 6-phosphogluconate dehydrogenase (*6-pgdh*, EC 1.1.1.44); phosphoglucomutase (*pgm*, EC 2.7.5.1); and sorbitol dehydrogenase (*sdh*, 1.1.1.14). Alleles at each locus were scored by designating the most common allele of *B. areolata* as 100. All other alleles were numbered according to their relative anodal distance from the reference allele. Chi-square goodness-of-fit tests were computed to determine if there were significant deviations from Hardy-Weinberg equilibrium between observed and expected heterozygote genotype frequencies at each locus (Nei 1978). The mean observed and expected heterozygosity in each population was also calculated (Nei 1978). Nei's genetic distance coefficients (D) were calculated and clustered by the unweighted pair-group method with arithmetic means (UPGMA) algorithm (Sneath and Sokal 1973). These analyses were performed with BIOSYS-1 (Swofford and Selander 1989).

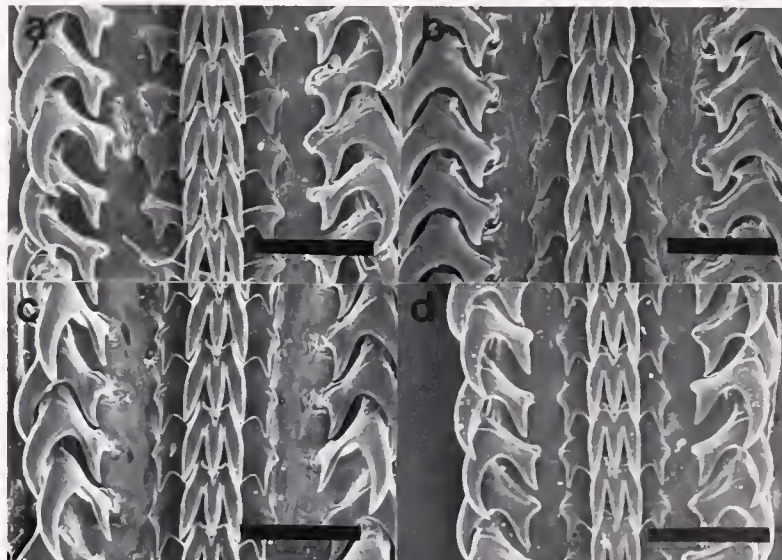


Figure 4. Micrographs of the radula of *Babylonia*: (a) male *B. areolata*; (b) female *B. areolata*; (c) female *B. formosae formosae*; and (d) female *B. formosae habei* (scale bar = 500 μm).

TABLE 3.
Allele Frequencies of *Babylania* Species.

Species/ Allele	<i>B. areolata</i>				<i>B. formosae formosae</i>		<i>B. formosae habei</i>	
Population	KH	TK	PH	PT	KH	TK	CC	PT
ARK								
(n)	30	28	30	27	55	50	68	30
189	0	0	0	0	0	0	0.118	0.100
156	0	0	0	0	0	0	0.875	0.900
100	0.917	1	0.950	1	0	0	0.007	0
89	0.083	0	0.050	0	0	0	0	0
56	0	0	0	0	0.982	0.900	0	0
40	0	0	0	0	0.018	0.100	0	0
Ho	0.100	0	0.100	0	0.036	0.040	0.221	0.200
He	0.155	0	0.097	0	0.036	0.182	0.222	0.183
	*					**		
EST-1								
(n)	30	28	30	27	55	50	68	30
100	1	1	1	1	1	0.990	1	1
94	0	0	0	0	0	0.010	0	0
Ho	0	0	0	0	0	0.020	0	0
He	0	0	0	0	0	0.020	0	0
EST-2								
(n)	29	28	30	27	55	48	67	30
111	0	0	0.017	0	0	0.031	0.015	0
100	0.983	0.982	0.766	0.981	0.991	0.948	0.948	1
89	0.017	0.018	0.217	0.019	0.009	0.021	0.037	0
Ho	0.003	0.004	0.433	0.037	0.018	0.063	0.075	0
He	0.003	0.004	0.371	0.037	0.018	0.101	0.101	0
						**	**	
GOT-1								
(n)	30	28	30	27	55	50	68	30
176	0	0	0	0	0.091	0.070	0	0
151	0	0	0	0	0.609	0.530	0	0
128	0	0	0	0	0.027	0.030	0.956	0.917
112	0	0	0	0	0.218	0.360	0.044	0.083
100	0.983	1	0.950	0.963	0.055	0.010	0	0
94	0.017	0	0.050	0.037	0	0	0	0
Ho	0.003	0	0.100	0	0.418	0.400	0.088	0.167
He	0.003	0	0.097	0.073	0.575	0.589	0.085	0.155
				**	**			
GOT-2								
(n)	30	28	30	27	55	50	66	30
160	0	0	0	0	0	0	0.030	0.017
100	1	1	1	0.944	1	1	0.955	0.983
40	0	0	0	0.056	0	0	0.015	0
Ho	0	0	0	0.111	0	0	0.091	0.033
He	0	0	0	0.107	0	0	0.088	0.033
IDH								
(n)	30	28	30	27	55	50	68	30
100	1	1	1	1	1	1	1	1
MDH								
(n)	30	28	30	27	55	50	68	30
115	0	0	0	0	0.082	0.060	0	0
100	1	1	1	1	0.918	0.940	1	1
Ho	0	0	0	0	0.091	0.040	0	0
He	0	0	0	0	0.152	0.114	0	0
					**	**		
MPI								
(n)	30	28	30	27	55	50	68	30
100	1	1	1	1	0	0	0	0

continued on next page

TABLE 3.

continued

Species/ Allele	<i>B. areolata</i>				<i>B. formosae formosae</i>		<i>B. formosae habei</i>	
93	0	0	0	0	1	1	0	0
76	0	0	0	0	0	0	1	1
OPDH								
(n)	30	28	30	27	55	50	68	30
100	1	1	1	1	1	1	1	1
6-PGDH								
(n)	29	28	30	27	55	50	68	30
150	0.034	0	0	0	0	0	0.015	0
100	0.966	1	1	1	1	1	0.978	1
67	0	0	0	0	0	0	0.007	0
Ho	0.007	0	0	0	0	0	0.044	0
He	0.007	0	0	0	0	0	0.044	0
PGM								
(n)	30	28	30	27	55	50	68	30
327	0	0	0	0	0.091	0.240	0.176	0.116
303	0	0	0.017	0.019	0.809	0.690	0.706	0.800
252	0	0	0	0	0.091	0.060	0.118	0.067
170	0.033	0	0.033	0.019	0	0.010	0	0.017
100	0.950	1	0.950	0.962	0.009	0	0	0
79	0.017	0	0	0	0	0	0	0
Ho	0.100	0	0.100	0.074	0.291	0.400	0.206	0.333
He	0.098	0	0.098	0.073	0.332	0.467	0.460	0.347
						**	**	
SDH								
(n)	30	28	30	27	55	50	68	30
100	1	1	1	1	1	1	1	1

(n: sample size.

Ho: observed heterozygosity.

He: expected heterozygosity. *, **: Significant deviation from Hardy-Weinberg proportion at $p = .05$ and $p = .01$, respectively).

RESULTS

Quantitative measurements of shell characters are indicated in Table 2. Shell lengths of the examined species were from 39 to 65 mm. Shoulder width was the only character shown significant

variation among species: *B. formosae habei* > *B. areolata* > *B. formosae formosae*, respectively.

No significant difference in the radulae was found among the *Babylonia* species and between radulae of male and female *B. areolata* (Fig. 4). The radulae belong to the rachiglossan type. The

TABLE 4.

Summary of Genetic Variation in *Babylonia* Species.

Species and Population	Number of Loci	Mean Number Alleles per Locus \pm SE	Percentage of Polymorphic Loci*	Mean Heterozygosity	
				Observed Mean \pm SE	Expected Mean \pm SE
<i>B. areolata</i>					
KH	12	1.5 \pm 0.2	16.7	0.028 \pm 0.012	0.032 \pm 0.015
TK	12	1.1 \pm 0.1	0.0	0.003 \pm 0.003	0.003 \pm 0.003
PH	12	1.5 \pm 0.2	33.3	0.061 \pm 0.036	0.055 \pm 0.031
PT	12	1.4 \pm 0.2	8.3	0.019 \pm 0.011	0.024 \pm 0.011
<i>B. formosae formosae</i>					
KH	12	1.8 \pm 0.4	25.0	0.071 \pm 0.040	0.093 \pm 0.052
TK	12	2.0 \pm 0.4	41.7	0.080 \pm 0.044	0.123 \pm 0.058
<i>B. formosae habei</i>					
CC	12	1.9 \pm 0.3	25.0	0.060 \pm 0.023	0.083 \pm 0.039
PT	12	1.6 \pm 0.3	25.0	0.067 \pm 0.036	0.068 \pm 0.039

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

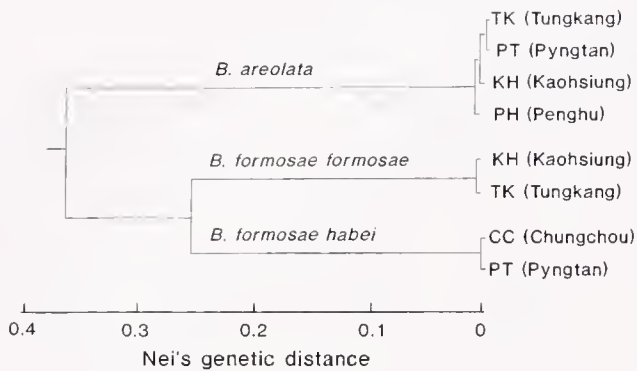


Figure 5. The UPGMA cluster analysis of Nei's (1978) unbiased genetic distance (D) among *Babylonia* species.

central tooth has five cusps. The lateral teeth have two curved cusps: the inner cusp short; and the outer cusp is long.

Among the 12 loci examined, using 0.95 as the criterion for polymorphism, six were polymorphic: *ark*, *est-2*, *got-1*, *mdh*, and *pgm*. Detailed allelic frequencies of *Babylonia* species are shown in Table 3. Fixed allelic differences were observed at *ark*, *got-1*, *mpi*, and *pgm* between *B. formosae* and *B. areolata* and at *ark*, *got-1*, and *mpi* between *B. formosae formosae* and *B. formosae habei*. Heterozygote deficiencies among populations and species were found in all the polymorphic loci. Mean heterozygosities among populations of *B. formosae formosae* and *B. formosae habei* varied from 0.060 to 0.080, which were higher than the populations of *B. areolata* (0.003 to 0.061) (Table 4).

Nei's genetic distance (D) between *B. formosae formosae* and *B. formosae habei* was 0.25. Comparison with other marine invertebrate genetic distance values, the difference between *B. formosae formosae* and *B. formosae habei* could be interpreted at a specific rather than a subspecific level. In addition, *B. areolata* was separated from *B. formosae formosae* and *B. formosae habei* at the distances of 0.35 and 0.37. An UPGMA cluster phenogram is shown in Fig. 5. Only minor differentiation existed among the populations in each of the three species ($D < 0.0002$ to 0.0030).

DISCUSSION

Our results indicated the shoulder width differed among species: *B. formosae habei* > *B. areolata* > *B. formosae formosae*, respectively. The fixed allelic difference between *B. formosae formosae* and *B. formosae habei* was observed in three of the 12 examined loci. The Nei's genetic distance between *B. formosae formosae* and *B. formosae habei* was 0.25. These allozyme differences are well above the specific level (Thorpe 1983; Richardson et al. 1986). Therefore, they should be recognized as two full species: *B. formosae* and *B. habei*.

By using the allozyme electrophoretic technique, several gastropod species previously considered to be polytypic or subspecies are actually separate species: *Oncomelania hupensis hupensis* and *O. hupensis quadrasi* (with Nei's genetic distance [D] = 0.62) (Woodruff et al. 1988), *Stramonita haemastoma canaliculata*, and *S. haemastoma floridana* (with D = 0.28) (Liu et al. 1991), *Nu-*

cella emarginata complex (with D = 0.16) (Palmer et al. 1990), *Crepidula convexa* complex (with D = 0.76) and *C. plana* complex (with D = 0.39) (Hoagland 1984). Although no simple relationship exists between genetic distance and taxonomic level, Thorpe (1983) found the Nei's genetic distances range from 0.19 to 2.59 for >95% of the congeneric invertebrates. Richardson et al. (1986) also suggested that fixed allelic difference can be diagnostic in separating species if the loci with fixed allelic differences are >20% of the examined loci. In the present study, Nei's genetic distance (D = 0.25) and the level of fixed allelic differences (25%) all indicated that the difference between *B. formosae formosae* and *B. formosae habei* is on the specific level.

According to the records of Altena and Gittenberger (1981), *B. formosae habei* are distributed on the northeast coast of Taiwan; however, we were unable to locate them. From a reliable record indicating the natural distribution of *B. formosae formosae* is on the southwest coast of Taiwan and that of *B. formosae habei* is on the southeast coast of China (Ke and Li 1991) (Ke and Li 1992). The occurrence of *B. formosae habei* in southern Taiwan was observed in 1987; since then it has been a very common shellfish in fish markets. It has become very rare after 1996, with one or two individuals mixed in hundreds of *B. areolata*. Because of this unusual change in abundance, we suspected that *B. formosae habei* might have been introduced from China through fisheries operations and is not a resident species in southern Taiwan. It is also speculated that range expansion could be caused by a temporal change in hydrographic condition.

Introduction of an exotic species either by an accident or commercial purpose is quite common in Taiwan. For example, *Perna viridis* was believed to be imported by ship industry (Lai 1987). A South America freshwater apple snail, *Ampullarius insularis* was imported from Argentina for commercial use in 1980 (Chang 1985). Both species are now widespread in Taiwan. In addition, marine bivalve *Mytilopsis sallei*, freshwater snail *Pila leopardvilensis*, and land snails *Achatina fulica* and *Bradybaena similaris* are also introduced species in Taiwan. The presence of *B. formosae habei* in Taiwan might be another case of an importation from southeast coast of China.

The reproductive season of *B. formosae formosae* is known to be from October to January, with average egg diameter 0.55 mm (Chiu and Liu 1994); whereas, *B. formosae habei* spawns from June to September with average egg diameter 0.26 mm (Ke and Li 1991) (Ke and Li 1992). The colors of the female sperm-ingesting gland are also different, being dark brown and brown, in *B. formosae formosae* and *B. formosae habei*, respectively (personal observation). Although the breeding compatibility of these two subspecies is unknown, the differences in allozyme patterns indicated they are different and should be elevated to full species level; that is, *B. formosae* and *B. habei*.

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CHARACTERIZATION OF THE DIGESTIVE TRACT OF GREENLIP ABALONE, *HALIOTIS LAEVIGATA* DONOVAN. I. MORPHOLOGY AND HISTOLOGY

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ABSTRACT Australasian abalone such as the greenlip abalone, *Haliotis laevigata*, prefer a diet of red algae (Rhodophyta); whereas, abalone from elsewhere more commonly prefer brown algae (Phaeophyta). Because of this feeding preference, the structure of the digestive tract of *H. laevigata* was investigated using histological and scanning electron microscopy (SEM) techniques. The digestive tract of both starved and fed adult *H. laevigata* revealed the presence of ciliated, mucus, and secretory cells throughout the digestive tract. The esophagus contained secretory, ciliated, and large mucous cells, with fragmentation spherules also present. The crop extended from the esophagus to the stomach. It was surrounded by thin muscularis and consisted mainly of secretory cells, although some phagocytes were present. The stomach possessed mainly secretory cells, although some ciliated cells, mucous cells, and phagocytes were present. The style sac differed from the stomach, having more ciliated cells. In intestinal regions I to III, the epithelium was shorter than in previous regions. Few cilia were present on the ridges, although many were observed in the gutters. Intestinal regions IV to V contained more mucous cells than intestine III, and more bacteria were observed associated with the fecal string than in other regions. The low incidence of bacterial association with the gut epithelium was attributed to the occurrence and number of mucous cells, common throughout the digestive tract. Spherical bodies present in the lumen are believed to be fragmentation spherules involved in waste removal and enzyme release. Starved abalone contained fewer mucous cells in the esophagus, had less pronounced staining reactions in the stomach, contained large amounts of granular inclusions in the style sac, and had fewer phagocytes in the intestines.

KEY WORDS: Abalone, *Haliotis laevigata*, histology, digestive tract, starvation

INTRODUCTION

Abalone are herbivorous archaeogastropods whose diet consists mainly of macroalgae, although diatoms and some detritus, including sand, are also ingested (Campbell 1965, Garland et al. 1985). Australasian abalone prefer red algae (Rhodophyta) and will consume brown algae (Phaeophyta) only when preferred species are less common (Poore 1972, Shepherd 1973, Shepherd and Steinberg 1992). Brown algae are digested much more slowly (Foale and Day 1992). Abalone from elsewhere more commonly prefer brown algae (Shepherd and Steinberg 1992), suggesting possible differences in digestive strategy. Several enzymatic studies of abalone from the northern hemisphere are available, but few data are available for Australasian species (Clark and Jowett 1978). Feeding preference differences for Australasian species have been attributed to algal toughness (McShane et al. 1994) or phenolic content (Shepherd and Steinberg 1992), although Shepherd and Steinberg (1992) considered that feeding preferences are primarily attributable to the selective nature of environments on available algae.

In such primitive gastropods as abalone, digestion begins with extracellular digestion followed by phagocytosis, or cellular uptake of particles, in the digestive gland and ingestion by mobile amoebocytes in some cases (Owen 1966). Intracellular digestion occurs within the duct cells of the digestive gland (Fretter and Graham 1962). Secretory cells occur throughout the digestive tract of *Haliotis* spp. They have been documented in the buccal cavity, crop, stomach, cecum, digestive gland, style sac, and intestine by Crofts (1929). Other locations for secretory cells include the buccal pouches (Fretter and Graham 1962) and esophagus (Bevelander 1988).

A wide variety of digestive enzymes have been identified from the gut of abalone with the carbohydrates fucoidan, carboxymethylcellulose (CMC), and algin being among the most common sub-

strates used for detecting enzyme activity (Duffas and Duffas 1968, Elyakova et al. 1981, Yamaguchi et al. 1989, Boyen et al. 1990). Protease, alginase, and amylase activity also appear in the crop fluid of *Haliotis rufescens* Swainson (McLean 1970).

The nature of the digestive tract of *Haliotis* spp. was examined by Crofts (1929) (drawings for *Haliotis tuberculata* Linnaeus), Campbell (1965) (drawings for *Haliotis cracherodii* Leach), and Bevelander (1988) (a photographic study for *H. tuberculata*). Examination of the greenlip abalone digestive tract, by histology and scanning electron microscopy (SEM), strengthens our knowledge of normal abalone gut structure, particularly for species that prefer red algae. It also complements current gut physiology research (Harris et al. 1998a). In addition, it facilitates the detection of dietary or toxicant-induced alterations in structure (Harris et al. 1998b).

Contributions to host animals from digestive tract bacteria can come from either resident or transient populations. Alimentary tracts offer many habitats conducive to microbial activities, such as fermentation of complex organic molecules, the products of which can be used by the host. Intestinal surfaces are often colonized by bacteria, which then make up the autochthonous flora of the host (Savage 1983), and which can contribute to the nutrition of the host (McBee 1971). Transient bacteria are ingested with, or as, food and encounter both physical or biological events during passage that protect the resident populations from displacement (Orpin and Anderson 1988). Scanning electron microscopy has been used on the oysters *Crassostrea virginica* Gmelin and *Crassostrea gigas* Thunberg to examine bacterial associations (Tall and Nautman 1981, Garland et al. 1982a) and has revealed physical attachment by resident microbes to internal digestive surfaces of other invertebrates (Harris 1993, Jolly et al. 1993).

In this study, the gut structure of greenlip abalone is characterized using histological and/or SEM techniques for fed and starved individuals. Because the digestive gland is the most com-

prehensively studied organ involved in digestion in abalone (Campbell 1965, McLean 1970, Bevelander 1988) and is known to be free of bacteria (Erasmus et al. 1997), it was not considered in this study. Our emphasis was on epithelial function, because this can influence bacterial associations with the gut wall. It complements a study of the gut microenvironment in this species (Harris et al. submitted).

MATERIALS AND METHODS

Adult specimens of *H. laevigata* were maintained in a recirculating system consisting of 6 × 20 L plastic containers and a biofilter for up to 3 weeks prior to histological sampling. Greenlip abalone were collected from several locations in northern Tasmania, 40° to 41°50'S, 146°50' to 148°50'E (Petal Point, Foster Islands, Port Sorell, and Flinders Island) and consisted of adults of 135 to 185-mm length. Macroalgae were collected in southern Tasmania, 42°50' to 43°50'S, 147°50' to 148°E (Blackman Bay and Port Arthur). Macroalgae of the genera *Polysiphonia* sp., *Ulva* sp., and other epiphytic algae associated with the macroalgae *Amphibolus* spp. were collected by divers and used as food. Epiphytic algae of seagrasses are known to comprise up to 85% of the greenlip abalone diet in wild conditions (Shepherd 1973). The algae were added to the maintenance tank and left for 10 to 14 days. The abalone fed rapidly when *Polysiphonia* sp. were added, although they were "messy" feeders. To remove algal debris, tanks were siphoned every second day. A diatom film, which was grazed by the abalone, developed within the tank during the study period.

Abalone were removed from the maintenance tank by either a commercial abalone iron, a warm water siphon or a flat plastic spatula with grease. Several abalone were dissected for preliminary investigation of the digestive tract. For histological examination, separate groups of fed and starved animals were used. No macroalgae were added for 10 days to tanks containing starved abalone, allowing sufficient time for physiological changes to occur within the abalone (Carefoot et al. 1993).

A scalpel was used to cut the foot as close as possible to the shell without disrupting the mantle and visceral mass. The mantle surrounding the mantle cavity was removed to facilitate access to the digestive tract, taking care not to puncture the rectum (intestine V). Two parallel incisions were made in the integument covering the digestive tract (Fig. 1), joined by an additional two longitudinal cuts in the integument to complete a rectangular section. This layer was peeled away from the digestive tract with scalpel, tweezers, and a blunt probe. The intestine (sections II, III, IV, and V) were teased with a blunt probe from the connective tissue and membranes, and excised with scissors and scalpel. Because the oesophagus lies under the intestines, it was carefully separated by teasing with a blunt probe. The digestive gland and gonad were removed by scraping them from the surface of the crop and stomach.

Short lengths of gut (about 10 mm) were removed from the esophagus, the crop, the stomach wall, the style sac, and intestinal regions III and IV and placed into either phosphate-buffered formalin, Zenker's fluid, or Bouin's fluid during daylight hours. Samples were fixed for 24 hours at room temperature (15 to 18°C) then dehydrated through a graded ethanol series to xylene in a Tissue-Tek II tissue processor. Dehydrated tissue samples were embedded in paraffin resin on a Shandon Histocentre 2 and sectioned on a Microm HM 340 microtome at 4 µm. Sections were oven dried overnight at 37°C. Routine Harris' Haematoxylin and

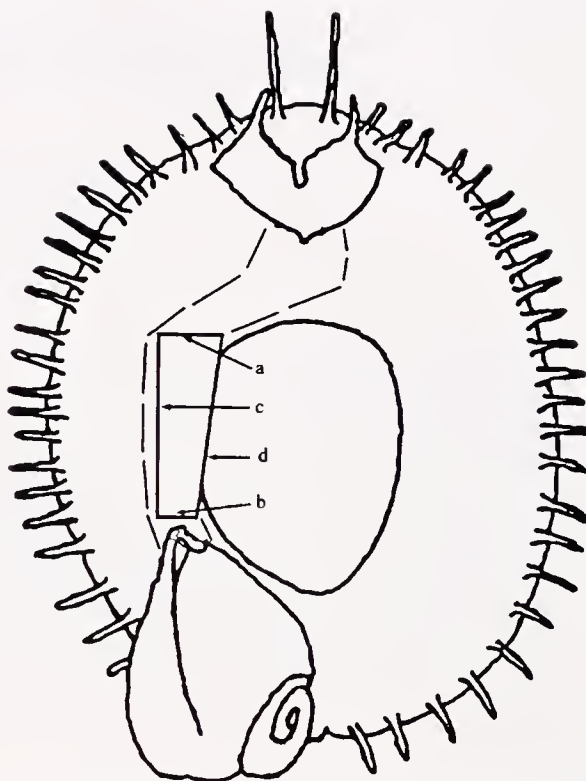


Figure 1. Schematic diagram of greenlip abalone showing locations for incisions into integument (a,b—lateral cut site; c,d—longitudinal cut site).

Eosin (H & E) staining in a Shandon Linistain GLX automatic tissue stainer was carried out on all tissues processed.

Five abalone, all of which had been maintained in recirculating aquaria and fed with red, filamentous algae *Polysiphonia* sp. were used for SEM. Abalone were removed from the shell, and the digestive tract was exposed. To preserve the contents, whole gut sections were removed aseptically. Where necessary, regions of the gut were teased from the integument with a blunt probe and/or forceps. Samples were taken from the esophagus, crop, stomach, style sac, and intestines III and IV. Samples were immediately transferred into 2.5% glutaraldehyde fixative in 0.2 M cacodylate buffer, pH 7.2 containing the major salts present in sea water: 1.6% NaCl, 0.6% MgCl₂, and CaCl₂ (Garland et al. 1982b). Samples were fixed for 24 h at 4°C then rinsed through three 0.1M cacodylate buffers of decreasing osmolality (Lewis et al. 1985). At this stage, the samples were trimmed and cut open to expose internal surfaces. The samples were then dehydrated through a graded ethanol series (Hodson and Burke 1994). Absolute ethanol and acetone were prepared by storing the commercial grade chemicals over anhydrous copper sulphate (in dialysis tubing). Dehydrated samples were immediately transferred to acetone prior to being critical point dried in liquid CO₂. Samples were dried using a Balzers CPD 020 Critical Point Dryer (CPD) apparatus. The samples were mounted onto aluminium SEM stubs with carbon paint and/or double-sided tape. Samples were then kept in a plastic desiccator and stored over CaCl₂ at a vacuum pressure of 25 to 30 psi, to prevent rehydration of the dried samples (Garland et al. 1982b), and sputter coated with gold (Balzers coater) within 24 h. Gut sections were viewed with a Phillips 505 SEM at operating

TABLE 1.

Cellular types distributed within the digestive tract of greenlip abalone, *H. laevigata*.

Gut Region	External Color	Cell Types Observed
Esophagus	Pale	Mucus, ciliated, and secretory
Crop	Blue-gray	Secretory, phagocytes
Stomach	Green-gray	Secretory, ciliated, mucus, phagocytes, amoebocytes, muscularis
Style sac	Green-gray	Secretory, ciliated
Intestine II	Light brown	
Intestine III	Gray-black	Ciliated, phagocytes, secretory
Intestine IV	Brown	Mucus, secretory, ciliated
Intestine V	White	

voltages between 15 to 20 kV, using Ilford FP4 120 film for micrographs.

RESULTS

The epithelium of the greenlip abalone digestive tract varied in shape, cellular types, composition, and staining reaction (Table 1). The efficacy of fixative type had a marked influence on cellular appearance between and within different regions of the gut. Starvation caused very minor effects to the epithelia of the esophagus, style sac, and intestines, with little effects noted elsewhere (Table 2). SEM of digestive tract epithelium revealed few bacterial cells, with most in the intestines. What is apparent from this investigation is the widespread occurrence throughout the digestive tract of spherical bodies, 5. to 8- μ m in diameter, corresponding to the fragmentation spherules described by Morton (1953), believed to be involved in waste removal and/or delivery of enzymes to the lumen. Although their size and spherical nature suggested the possibility of these spherules being bacterial, visible evidence of spherules being shed into lumen from the crop (see Fig. 7) and style sac (see Fig. 13) indicates otherwise. A general plan of the digestive tract of *H. laevigata* was developed from the morphological observations (Fig. 2).

Esophagus

The esophagus of the greenlip abalone is oriented posterior to the cephalic region. The entrances to the esophageal pouches lie immediately posterior to the salivary glands. The right esophageal pouch is twisted over the esophagus, following the rest of the digestive tract posteriorly, to the region where intestine III begins.

TABLE 2.

Changes in the digestive epithelium of starved greenlip abalone, *H. laevigata*, as indicated by histology.

Tissue	Starved
Esophagus	Fewer mucous cells on ridges
Crop	No difference
Stomach	Less pronounced staining
Style sac	Large amounts of granular inclusions
Intestine II	Fewer phagocytes
Intestine III	No difference
Intestine IV	More intense staining
Intestine V	No difference

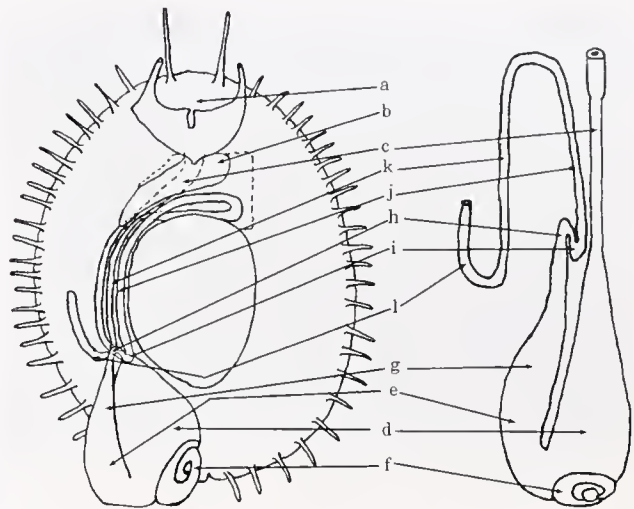


Figure 2. General layout of greenlip abalone digestive tract, showing (left) digestive tract in situ, and (right) schematic diagram of the digestive tract, in dorsal view (a = cephalic region; b = right buccal pouch; c = esophagus; d = crop; e = stomach; f = stomach cecum; g = style sac; h = intestine I; i = intestine II; j = intestine III; k = intestine IV; l = intestine V). The esophagus and crop are equivalent to the postesophageal regions I and II as described in Campbell (1965).

The left esophageal pouch is smaller and is located underneath the right esophageal pouch. The midesophagus extends to the posterior end of the esophageal pouches, where it then becomes paler and continues to the crop. This paler section corresponds to the post-esophagus region I of *H. cracherodii* (Campbell 1965), but is referred to as the esophagus in this study. No muscularis was observed in this region. The oesophagus contains mucous cells, secretory cells, and ciliated cells (Fig. 3). Large mucous cells occur on the ridges of the esophagus, among secretory cells containing granules. Granular inclusions occurred in the distal cell tips of both fed and starved animals. Ciliated cells occurred on both the ridges and gutters. Comparison with starved abalone revealed many more mucous cells on the ridges of the esophageal cells of fed animals. Within the esophagus, fragmentation spherules were evident among mucus within the lumen (Fig. 4). Neither the gut wall nor ingested food showed bacterial association. Food particles were associated with the mucus (Fig. 5).



Figure 3. Esophageal epithelium from fed abalone in histological section; tissue was fixed in Zenker's fluid; magnification 400 \times (a = granular inclusions; b = ciliated epithelium; c = mucous cell).

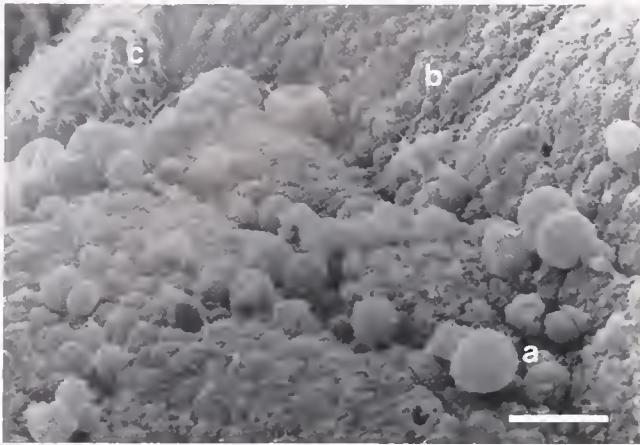


Figure 4. Epithelial surface of esophagus using SEM; Bar = 10 μ m (a = fragmentation spherules; b = mucus; c = cilia).

Crop

The posterior oesophagus I expands into the crop, which is distinguished by its deep blue-gray color. The crop extends to the most posterior point of the right foot muscle, then narrows and twists 180° into the stomach. Two large folds extend from the crop into the stomach, forming a valve that controls entry of material into the stomach. Thin muscularis surrounded the crop. The epithelium of the crop contained mainly columnar secretory cells, although phagocytes were also present (Fig. 6). Observed within the crop were cells in various stages of constriction and fragmentation, demonstrating the stages involved in release of fragmentation spherules (Fig. 7), including bulging through the mucus. The cells' surface also had a striated border. Granular inclusions were prevalent toward the distal cell tips in both fed and starved animals. Nuclei were mainly located in the basal half of the cells. Heavily ciliated regions contained both debris and fragmentation spherules (Fig. 8). In the crop, greater cell definition was found with Zenker's fluid than other fixatives. An isolated helical bacterial cell was observed in this region.

Stomach

The stomach of *H. laevigata* follows the general pattern for prosobranch gastropods as described by Morton (1953). A gastric

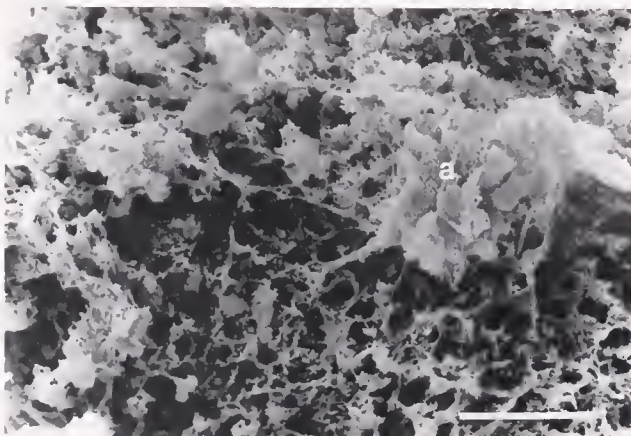


Figure 5. Esophageal epithelium, showing mucopolysaccharide matrix and algal fragments using SEM. Bar = 50 μ m (a = algal fragments in mucus).



Figure 6. Crop epithelium of starved abalone in histological section; tissue was fixed in Zenker's fluid; magnification 400 \times (a = nucleus of mucus cell; b = non-nucleated fragmentation spherules; c = muscularis).

shield is present on the anterior, right side wall. A furrowed ciliary sorting area takes up the floor of the stomach. The stomach chamber narrows anteriorly into the heavily ciliated style sac, in which is located the protostyle. The style sac continues to narrow into the intestine. The ducts known to lead to the digestive gland in other species of *Haliotis* were difficult to locate in *H. laevigata*, although these ducts are presumably present. Muscularis was also found below the epithelial cells. Cells comprising the stomach epithelium included ciliated cells, mucous cells, secretory cells, and phagocytes (Fig. 9). Secretory cells constituted the majority of the epithelium, with mucous cells occurring consistently. Phagocytes, although infrequently observed, were present both in basal and distal regions of the cells. Nuclei were located within the basal third of the cells. Amebocytes occurred in the stomach epithelia and were distinguishable from phagocytes by their irregular morphology. Some cilia were also visible under the spherules (Fig. 10). Bulging of the secretory cells through the epithelium and cavities in the mucus occurred in the style sac (Fig. 11), similar to the observations of the crop. Secretory cells involved in fragmentation appear uniform in view from the epithelial surface, but side views of fragmented cells indicate more variety in shape. The club-shaped tips of the cells were observed protruding from the

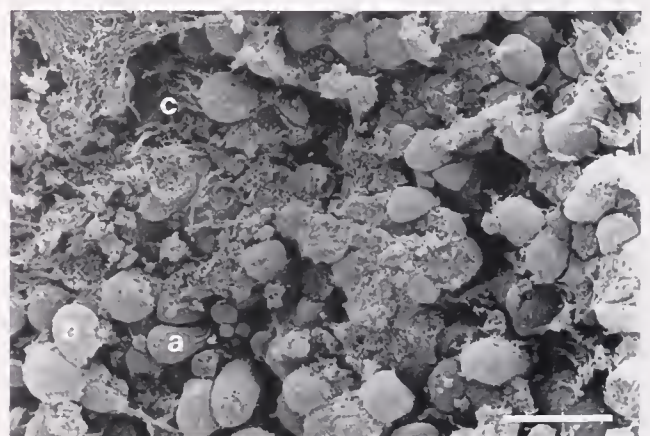


Figure 7. Epithelium of crop wall, showing secretory cells in various stages of constriction and fragmentation using SEM; Bar = 10 μ m (a = constricting cell; b = fragmented cell; c = cilia).

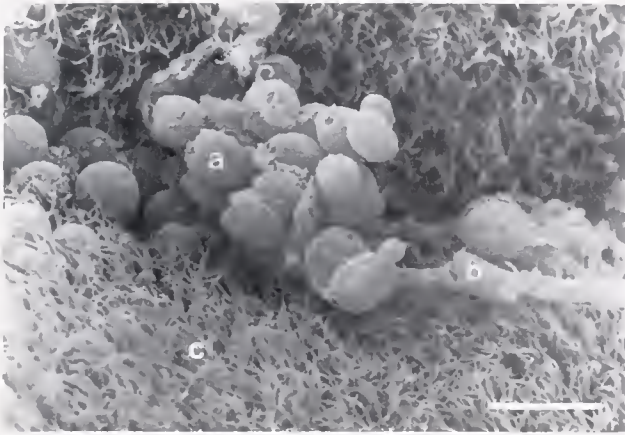


Figure 8. Crop epithelium, showing a region where cilia are located. Fragmentation spherules are collected together in a furrow of the epithelium using SEM. Bar = 10 μ m (a = fragmentation spherule; b = algal debris; c = cilia).

mucus. The style sac has an evenly ciliated, striated epithelium (Fig. 12), although epithelia toward the stomach showed fewer cilia and more secretory cells. Bouin's fluid produced more definition in cellular structure than other fixatives, although formalin-fixed tissue enabled phagocytes to be distinguished more easily. Interestingly, formalin-fixed stomach tissue retained a large layer of mucus that also showed separation from the epithelial surface. In starved animals, tissue anterior to the stomach showed large amounts of granular inclusions within the distal third of the cells and in the striated borders. Nuclei of starved tissue cells also showed a less pronounced staining reaction (Figs. 13).

Intestines I to III

Intestine I continued from the narrowed style sac, crossed the oesophagus dorsally, into the first of the vertically oriented 180° intestinal twists. The second 180° twist and a change in exterior color to light brown/beige marked the beginning of intestine II, which extended to about the midpoint of the foot. Here the intestine abruptly changed exterior color to almost black, indicating the start of intestine III. No muscularis was observed in this region.

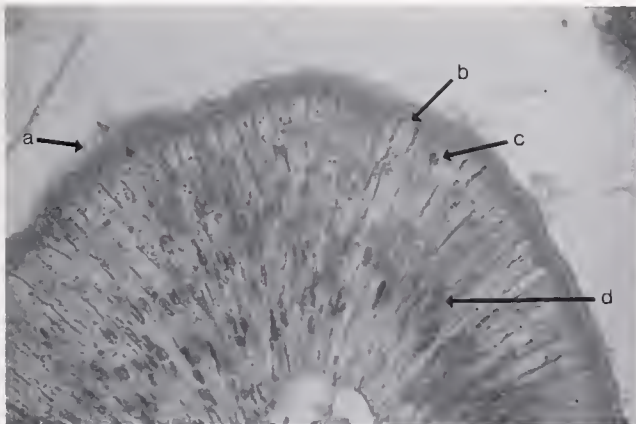


Figure 9. Stomach epithelium of fed abalone in histological section; tissue was fixed in Bouin's fluid; magnification 400 \times (a = fragmentation spherule; b = club-shaped tip of secretory cell bulging towards lumen; c = phagocyte; d = nucleus of secretory cell).

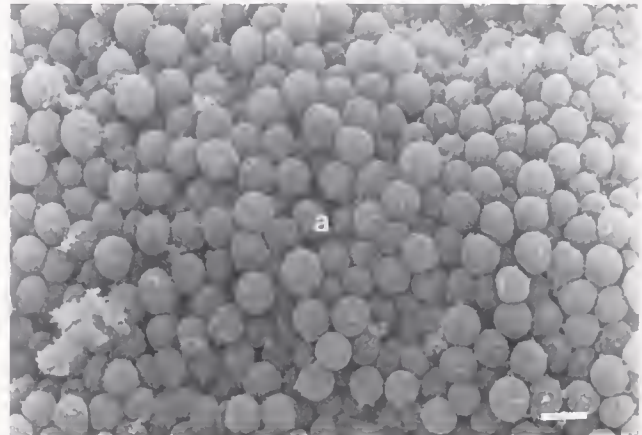


Figure 10. Epithelial surface of the left stomach wall using SEM. Note the occurrence of secretory cells involved in fragmentation as the dominant cell types. Bar = 10 μ m (a = fragmentation spherules; b = cilia).

The epithelium was most similar to that of the crop. Cells within intestine III were more cuboidal than in intestines I and II. Few ciliated cells were present on the ridges (Fig. 14), though more occurred in the gutters, and were observed supporting fragmentation spherules within the intestine III. Phagocytes were widespread among the cells, although mainly confined to the distal cell tips. Formalin-fixed tissues revealed more mucous cells in ridge regions than other fixatives, as well as phagocytes located in the distal parts of the cells. Few phagocytes were evident within the starved tissue samples (Fig. 15), although several phagocytes occurred in the fed tissue samples. SEM revealed large areas of the epithelium covered by fragmentation spherules, and a layer of mucus. Where this layer of mucus was removed from the intestinal surface, the fragmentation spherules were apparent, indicating mostly secretory cells as the dominant cell type (Fig. 16). This removal of mucus may have been an artifact of the glutaraldehyde fixation process, but it shows the arrangement of the cells beneath the mucous coat. Epithelial cells can be seen in cross section where tissue was trimmed after fixation (Fig. 17). These cells were overlaid with mucus, some debris, and fragmentation spherules. On epithelial surfaces, more fragmentation spherules were found, along with rod-shaped bacteria (Fig. 18).

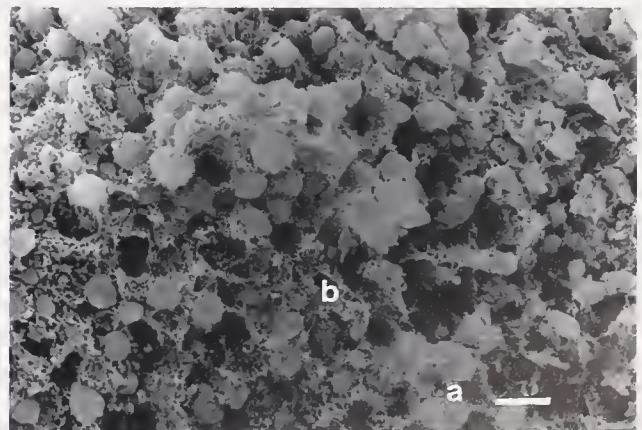


Figure 11. Epithelium of posterior style sac, showing mucous-associated secretory cells using SEM. Bar = 10 μ m (a = secretory cells; b = mucus).



Figure 12. Style sac epithelium of fed abalone in histological section; tissue was fixed in Bouin's fluid; magnification $400\times$ (a = ciliated cells).

Intestines IV to V

The intestine continues anteriorly to the most anterior point of the foot, where intestine IV begins. The exterior of intestine IV was mostly light brown in color. The intestine turns 180° and follows the previous regions of the gut, dorsal to intestine III and the esophagus. This continues to close to the intestinal bends (intestines I and II). Intestine V is defined by another 180° turn, passing through the ventricle to end within the mantle cavity as the white-colored rectum. Intestine IV contained more mucous cells than samples from intestine III (Fig. 19). Secretory cells and ciliated cells were also widespread, although ciliated cells were more common within some gutters. Cells within the gutters were also more cuboidal than those occurring in ridges (Fig. 20). Bouin-fixed tissues had more defined nuclei than either Zenker's or formalin-fixed tissues. In formalin-fixed samples, starved abalone tissues stained more intensely than fed abalone tissues. Granular inclusions appeared more prevalent within starved tissue samples. Some bacterial cells were evident, associated with the mucus of this region. These were mostly rod-shaped cells, and were few in number. These cells all appeared associated or entangled with the preserved mucus of the fecal string.

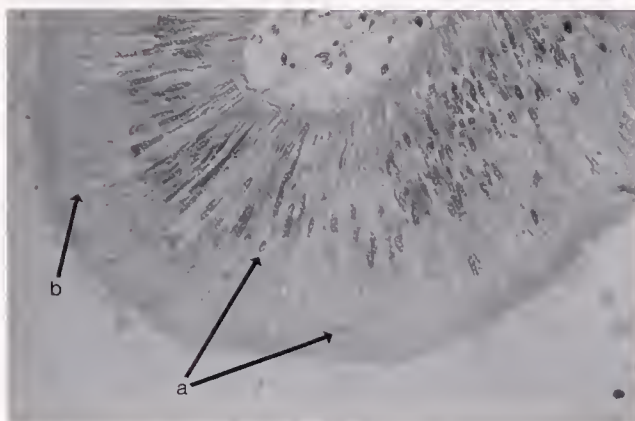


Figure 13. Style sac epithelium of starved abalone in histological section; tissue was fixed in phosphate-buffered formalin; magnification $400\times$ (a = phagocytes; b = granular inclusions).

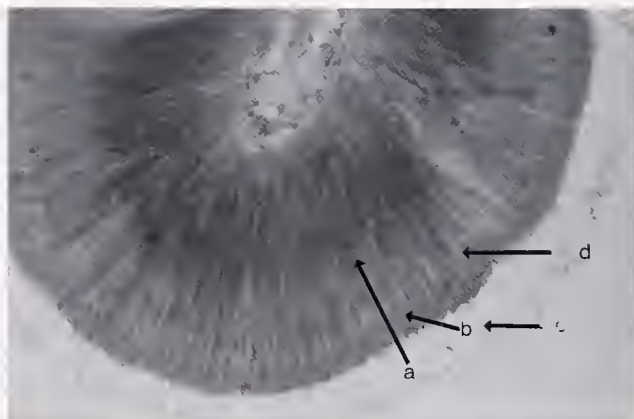


Figure 14. Intestine III epithelium from fed abalone in histological section; tissue was fixed in Bouin's fluid; magnification $400\times$ (a = nucleus of ciliated cell; b = ciliated cell; c = cilia; d = mucous cell).

DISCUSSION

Gut Structure

Cellular type and location within the digestive tract of *H. laevigata* illustrate digestive processes occurring in discrete regions. The presence of secretory, mucous, and ciliated cells in the epithelium of different regions enabled functions for each organ to be postulated. Distinguishing between secretory cell types requires further histological investigation of the cells and the roles they play in digestion. Each fixative considered here proved effective at highlighting certain structural details in particular areas. No general pattern emerged as to which fixative was suited to each selected area, although Bouin's fluid seemed best at fixing tissues of the stomach and style sac. Formalin-fixed tissues showed a more intense staining reaction, sometimes making subtle features, such as cytoplasmic granules, difficult to see.

The frequency and large size of mucous cells found within the esophagus indicate that this region is responsible for substantial mucus production. Mucous secretions aid in entangling food particles, thus enhancing movement of food through this region and into the crop. Food movement through the esophagus is also fa-

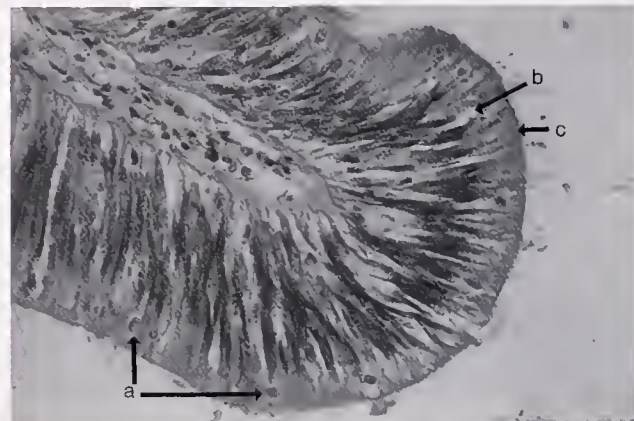


Figure 15. Intestine III epithelium of starved animals in histological section; tissue was fixed in phosphate-buffered formalin; magnification $400\times$ (a = phagocytes; b = mucous cell; c = ciliated cell).

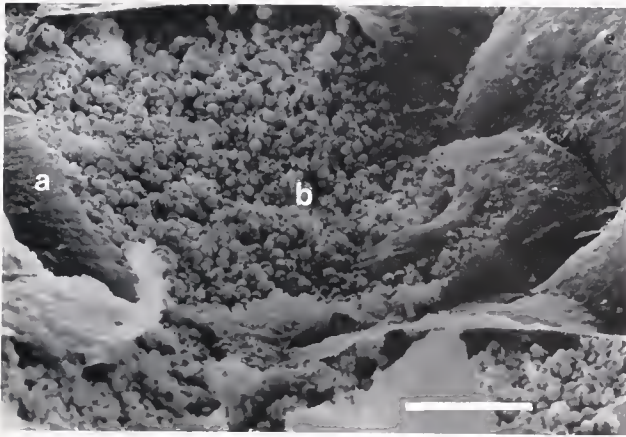


Figure 16. Ventral surface of intestine III using SEM. Bar = 100 μ m (a = mucus; b = epithelial surface with large numbers of secretory cells).

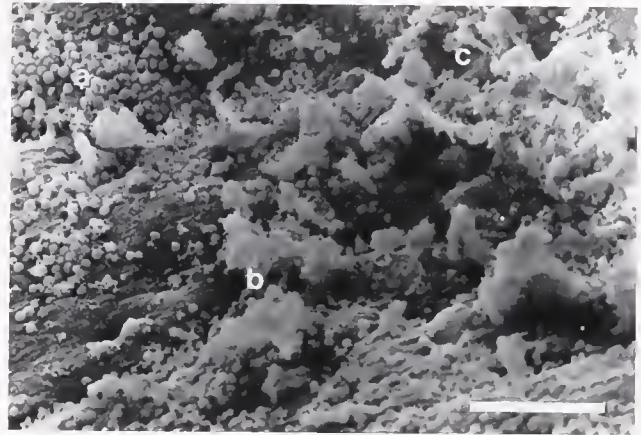


Figure 18. Epithelial surface of intestine III using SEM; note the occurrence of fragmentation spherules, debris, and some rod-shaped bacteria. Bar = 50 μ m (a = fragmentation spherules; b = fecal debris; c = rod-shaped bacterial cells).

ciliated by widespread cilia. The similar incidence of mucous cells in the intestinal region relates to the need for abalone to have cohesive fecal pellets, because feces are discharged directly into the mantle cavity, where disintegration of feces would be disadvantageous. Mucous secretion thus serves two purposes: it helps to lubricate passage of the fecal string, and it coats the fecal pellets in mucus that becomes more viscid with increasing pH typical of seawater (Morton 1968).

Large numbers of secretory and mucous cells were present within the crop, but very few ciliated cells were observed. The physical nature of the esophageal folds within the crop prevent uncontrolled food movement from the crop into the stomach. Because the crop receives the contents of the esophagus, including food, mucus, and secretory cell products, and contains few cilia, it seems likely that its function differs from the oesophagus. The internal pressure of the crop causes rapid leakage of gut contents if any part of its surface is punctured. Therefore, food is likely to be retained within the crop for some time, which, together with the expanded walls of the crop, suggests that it is a storage organ, although there may be some food digestion before the food-mucus mixture enters the stomach. Analysis of crop contents has revealed algal degradation within the crop (Shepherd 1973, Foale and Day

1992), implying some enzyme production to degrade algae beyond the particle size rasped into the digestive tract by the radula. Amylases have been recorded from the esophageal epithelium of *H. rufescens* (McLean 1970).

The large number of secretory cells observed in the crop and stomach suggest that these regions are important in digestion by *H. laevigata*. Crofts (1929) observed secretory cells in the crop, stomach, and digestive gland of *H. tuberculata*. Fragmentation spherules were widespread in the esophagus, crop, stomach, style sac, and intestine of all animals examined. These cells are not believed to be bacterial, because "pinching-off" of cells within the crop and style sac was observed in this study and by Campbell (1965) in *H. cracherodii*. These cells bulge into the lumen, becoming rounded and club-shaped and surrounded by mucous-bound food (Morton 1953). The tips of the cells, or whole cells, are constricted into the lumen. In this study, spherules were observed still intact in the intestine, supporting one view held by Owen (1966) that these spherules aid in removing indigestible wastes. The presence of spherules in the esophagus is unlikely to be associated with waste removal, so it is more likely that they carry digestive enzymes into the lumen to facilitate extracellular digestion. Breakdown of these spherules would, thus, be a means of introducing enzymes to the

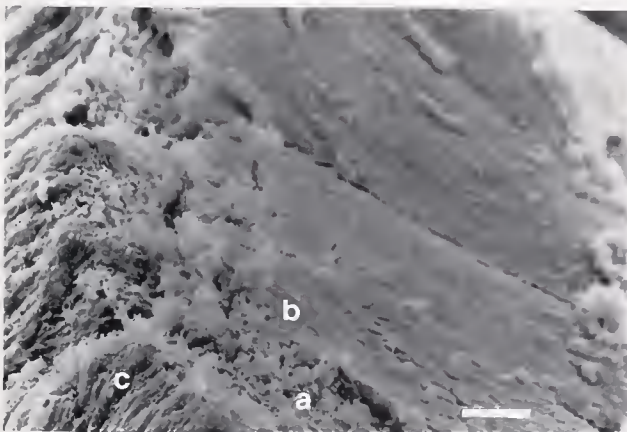


Figure 17. Intestine III, showing both a cross section of the epithelial cells and the surface mucus coating using SEM. Bar = 50 μ m (a = fragmentation spherules; b = mucus; c = epithelial cells).

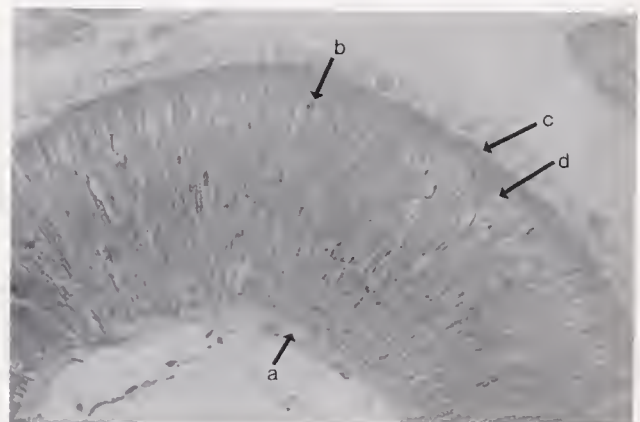


Figure 19. Intestine IV epithelium of fed abalone in histological section; tissue was fixed in Zenker's fluid; magnification 400 \times (a = basal lamina; b = phagocyte; c = ciliated cells; d = mucous cell).



Figure 20. Intestine IV epithelium of fed abalone in histological section; tissue was fixed in Bouin's fluid; magnification $400\times$ (a = ciliated cells; b = nucleus of ciliated cell).

large substrate volume of the food mass. Further definition of the role of spherules is required.

The widespread occurrence of cilia within the style sac and the presence of a protostyle within this region are typical features of gastropods (Morton 1953). The protostyle is known to receive indigestible wastes from the stomach sorting area and, because of the rotating action of cilia, bind these wastes onto the protostyle surface (Morton 1953). The extensive ciliation in the style sac suggests that *H. laevigata* has a stomach structure typical of other primitive archaeogastropods, such as *Patella* spp. (Fretter and Graham 1962). The high incidence of secretory cells would contribute to the digestive processes involved with the action of the protostyle, because chemical breakdown would greatly enhance the mechanical action of the protostyle on the gastric shield.

The presence of phagocytes near the distal epithelial cell tips in the stomach suggests a role in either waste rejection or food uptake (Owen 1966). When comparing intestine III to the esophagus, fewer cilia are visible. In view of this, food passage through intestine III would be much slower, allowing ample time for phagocytes to interact with the lumen contents. It would follow that this region has a role in food absorption and waste rejection. Because fewer phagocytes were recorded in the stomach and intestine of starved abalone, this could be seen as a response to decreased food availability. Intestinal region IV has distribution of cilia similar to intestine III. Thus, slow passage time through the intestines provides both ample time and surface area for either absorption or phagocytosis. The presence of granules in epithelial cells alludes to digestive activity, instead of simply the consolidation of the fecal string, as suggested by Campbell (1965).

Starvation of the abalone produced changes in some of the digestive tissues. Compared to fed animals, periods of starvation are known to decrease the rate of digestion of algae by *H. rubra* after feeding recommences (Foale and Day 1992). Both blood glucose levels and stored glycogen content were depleted within 6 days of starvation (Carefoot et al. 1993). Similar responses in cellular structure have been elicited by other causes, such as poor water quality (Harris et al. 1998b). Therefore, the structural changes could have several possible explanations.

Microbial Aspects

Overall, few bacteria were observed within the digestive tract of *H. laevigata*. Areas likely to be harboring microorganisms, such

as folds and crevices (McBee 1971), showed little evidence of colonization. In this study, most bacteria were evident in the intestine of *H. laevigata*. The isolated spirilloid bacterium observed within the crop and the several rod-shaped bacteria observed within the intestine all seemed to be associated more with mucus than directly with epithelial surfaces. Interestingly, only in the intestine were any bacteria observed that could be regarded as attached. These were located on the epithelial surface with fragmentation spherules and debris. Because this was only observed in one abalone, it suggests that bacterial attachment to intestinal surface may occur, but not to any great extent.

Mucus was observed closely associated with the epithelial surfaces, including ciliated surfaces. Detachment of some mucus in the intestine showed the surface of secretory cells with and without mucus; in neither case were bacterial associations evident. The presence of mucus after glutaraldehyde fixation is in contrast to Garland et al. (1979), who found tissue surfaces relatively free of mucus when fixed in this way. The secretion of copious quantities of mucus is believed to remove microorganisms from epithelial surfaces (Garland et al. 1979). Prosobranchs such as abalone secrete large amounts of mucus throughout the digestive tract, mainly from the esophageal glands and style sac (Crofts 1929, Fretter and Graham 1976). Mucus within *C. gigas* had an effect on microbial attachment, because of its viscosity, by entangling microbes and preventing their attachment (Garland et al. 1982a). The few bacterial cells observed among mucus in the intestines and esophagus of *H. laevigata* are unlikely to be attached populations, because microbes on the surface of a mucous layer are more likely to be originate from either food or feces (Harris 1993).

The few food particles observed in the esophagus of *H. laevigata* also revealed no bacteria, and only an isolated bacterial cell in one esophagus sample. The absence of bacteria in this region requires explanation, because abalone are known to consume microorganisms with ingested algae (Garland et al. 1985). The visible absence of bacteria may be attributable to the rapid passage time of food into the crop, because ciliary currents move food quickly through the esophagus of *H. tuberculata* (Crofts 1929). Movement of ingested, radio-labeled food into the blood of *H. rufescens* occurred within 15 minutes of feeding (McLean 1970), suggesting that movement of food and absorption through this region can be rapid. Because abalone feed soon after dusk (Shepherd 1973), food may be in the guts for up to 12 hours before collection, allowing adequate time for algae to fragment to an unrecognizable state (Foale and Day 1992). This may have influenced the results; however, the presence of isolated food particles within the oesophagus suggests other reasons for the absence of visible bacteria.

The lack of bacteria observed in this study within the gut of *H. laevigata* could be attributed, in part, to the physiological aspects of digestion associated with the three main cell types. Within the digestive tract, *H. laevigata* has a large surface area taken up by secretory, ciliated, or mucous cells. Mucous and secretory cells, through secretion, provide a surface area that would be unsuitable for microbial association (Harris 1993). Surfaces covered by cilia are unsuitable for microbial attachment, because it is through the movement of cilia that food is directed through all gut regions except the crop and stomach (Crofts 1929, Campbell 1965). The major means of food passage through the digestive tract of oysters is entanglement in mucus, directed by ciliary movement (Garland et al. 1982a). These authors found few bacteria within the digestive tract of the oyster *C. gigas* and attributed this to the extensive ciliation within the oyster.

The greenlip abalone seems to have a transitory relationship with its ingested bacterial populations, similar to *C. gigas* (Garland et al. 1982a). The nature of the gut of *H. laevigata*, with few visible bacterial associations, suggests that for possible bacterial contribution, bacteria within the food may be more important at breaking down carbohydrates than surface-associated bacteria. Both these possibilities have been suggested for other aquatic invertebrates (Harris 1993). Because bacterial populations within the lumen may be either transient populations or colonizers, significant contributions from these populations would require prolonged retention of food within the latter regions of the gut. Because feces are produced for several days postfeeding (Wee et al. 1992), this situation is entirely possible.

CONCLUSION

The gut structure of *H. laevigata* does not differ greatly from that of other haliotids that chiefly consume brown macroalgae. It has a complex gut with different regions being characterized by specific cellular composition indicative of region-specific gut

functions. SEM analysis suggests that relatively little of the gut epithelial surface is suitable for colonization by bacteria. The contribution of transient bacteria in the lumen of the gut could not be assessed in this descriptive study, but the contributions of bacteria to particular aspects of digestion are addressed in a complementary physiological study (Harris et al. 1998a).

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CHARACTERIZATION OF THE DIGESTIVE TRACT OF GREENLIP ABALONE, *HALIOTIS LAEVIGATA* DONOVAN. II. MICROENVIRONMENT AND BACTERIAL FLORA

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ABSTRACT Microelectrodes were used to measure pH and dissolved oxygen within the gut environment of adult greenlip abalone (145 to 160 mm), *Haliotis laevigata* Donovan. Oxygen levels were found to be below the limit of detection for the oxygen micro-electrode (0.38 mg DO.L⁻¹), suggesting either microaerophilic or anaerobic conditions. The pH profile of the gut revealed a decrease from the external environment (pH = 8.20) to pH 5.31 within the crop, increasing through the intestine to 6.64 in the rectum. Enrichment cultures of bacteria from within the abalone gut revealed mostly isolates from the family Enterobacteriaceae. These isolates occurred throughout all regions of the abalone gut, and almost all showed hydrolytic ability for one or more carbohydrates. *Cytophaga* spp. isolates appeared from esophageal and intestinal enrichments of the digestive tract and were all capable of both carboxymethylcellulose and agar hydrolysis. A decrease in diversity of bacterial types in the stomach, crop, and style sac corresponded with reduced pH.

KEY WORDS: Abalone, *Haliotis laevigata*, digestive tract

INTRODUCTION

The gastrointestinal tract is a microenvironment that has been examined in marine invertebrates in terms of pH (Mathers 1974) and dissolved oxygen or redox potential (Plante and Jumars 1992). As well as having a significant effect on enzyme activity, high or low pH can favor maintenance of symbiotic microbes (Plante and Jumars 1992). The selective nature of the microenvironment directly influences microbial composition and activity. In turn, it is possible for the microorganisms, through their metabolism, to modify the microenvironment.

The most commonly reported association between microbes and invertebrates involves the ingestion of bacteria (e.g., Garland et al. 1985, Vitalis et al. 1988). Bacterial associations with digestive tracts of marine animals have revealed a restricted range of microorganisms, suggesting the existence of strong selective pressures within the gut that result in characteristic gut microflora (Unkles 1977, Sochard et al. 1979, Tall and Nauman 1981). Studies on the bacterial flora of digestive tracts require an understanding of the microenvironment in order to mimic these conditions in culture. Microelectrodes are ideally suited for studying, *in situ*, some aspects of the physiology of undisturbed microbial communities, such as microbial respiration (Revsbech and Jørgensen 1986).

Nearly all terrestrial herbivorous animals have one or more parts of the digestive tract expanded into an organ that accommodates a microbial population valuable in the digestion of food, for which the host animals do not necessarily produce the correct complement of enzymes (McBee 1971). The activity of such bacteria often benefits the host through cellulose breakdown, nitrogen fixation, increased host resistance to toxins, or preconditioning of food (Harris 1993). In some herbivorous animals, there exists a specific fermentation organ, in which food (cellulose) is subjected to highly reduced conditions arising as a result of microbial metabolism. Abalone are aquatic herbivores. If an analogy can be drawn between herbivorous animals that ferment and abalone in terms of microbial cellulase activity, then the conditions prevalent in these fermentation chambers should also be repeated. These conditions would include a highly reduced environment in which oxygen has been removed. The most effective means of detecting

these conditions is with microelectrodes because of their small size, accuracy, and sensitivity. Oxygen depletion as seen within the digestive tract of vertebrates does occur in some aquatic deposit feeders (Plante and Jumars 1992).

Less is understood about the relationships that microbes have with invertebrate hosts than with vertebrates, with the exception of the cellulose-degrading bacteria found within Teredinidae (Bivalvia) (Morton 1978). Associations between microbes and aquatic invertebrates have been reviewed by Harris (1993). If not digested, microbes can travel the length of the gut and pass out unaffected, or may proliferate in a favorable region of the gut. Attachment often leads to the development of residential populations. The importance of the role these microbes play is unclear. Vitalis et al. (1988) found that bacteria that degraded algae contributed significantly to the nutrition of the sea-hare, *Aplysia* spp. However, Galli and Giese (1959) described several isolates from within the gut of the herbivorous aquatic snail, *Tegula funebris*, few of which could degrade either agar, alginic acid, or carrageenan.

Algal carbohydrates (Table 1) have been used as substrates to examine the role of microbes in aquatic herbivore digestive physiology. Alginate lyase, amylase, cellulase, agarase, laminarinase, carrageenase, and β -1,4-glucanase activities have all been evaluated (Galli and Giese 1959, Vitalis et al. 1988, Harris 1993, Sawabe et al. 1995, Erasmus et al. 1997). Bivalves that exhibit cellulase activity have been shown to possess a cellulolytic microflora (Crosby and Reid 1971). Other bacterial strains isolated from aquatic invertebrate guts have shown agarase, protease, lipase, laminarinase, amylase, alginase, and chitinase activities (Harris 1993).

Most commonly, facultatively aerobic bacteria have been identified from the digestive tracts of invertebrates (Galli and Giese 1959, Prim and Lawrence 1975, Musgrove 1988). However, few attempts to isolate strict anaerobes have been made (Harris 1993, Sawabe et al. 1995). In vertebrates, facultative aerobes are present, but in lower numbers than other types. The activities of these facultative aerobes quickly deplete the oxygen within the digestive tract, thus providing favourable conditions for growth of obligate anaerobes.

TABLE 1.

Common polysaccharides found in some marine algae (Kreger 1962, McCandless 1981, Craigie 1990).

Algal Division	Polysaccharides	
	Storage	Structural
Rhodophyceae	Floridean starch	Cellulose, agar, carrageenan, mannans
Chlorophyceae	Starch	Cellulose, xylans
Phaeophyceae	Laminarin	Cellulose, alginic acid, fucoidan

In aquatic invertebrate guts, bacteria can occur within the esophagus, the stomach, intestine, midgut, style sac, cecum, and hindgut (Harris 1993). In bivalves, the hindgut is the most heavily colonized region (Harris 1993). Accumulation of bacteria in the hindgut of bivalves occurs because of the extended passage time of food (Prieur et al. 1990). Bacteria have doubling times that range from 15 minutes up to several days, so passage times of up to 3 days through bivalve guts are sufficient for adapted bacteria to survive and grow (Prieur et al. 1990). Observations of blacklip abalone, *H. rubra*, indicate that feces are produced up to 7 days after feeding (Wee et al. 1992), suggesting ample time for bacterial colonization. Similarly, the relatively long intestine in abalone, with numerous folds and grooves (Campbell 1965), provides ample surface for bacterial colonization (Harris 1993). However, Harris et al. (submitted) found relatively few bacteria within the gut of *H. laevis*.

The abalone digestive tract contains several functional regions through which there is a continuous, one-way flow of ingested material (Harris et al. submitted). Characterization of the microenvironment of these areas would enhance understanding of the microbial and physiological processes occurring within the digestive system of abalone. The nature of the microbes from the greenlip abalone, *H. laevis*, also requires characterization to determine their potential importance to the digestive physiology of the host. The purpose of this study is to determine the physical conditions within the gut of the greenlip abalone and to examine the ability of microbes isolated from the gut to digest algal carbohydrates. This information complements another study on the gut structure of this species (Harris et al., submitted).

MATERIALS AND METHODS

Maintenance System

Adult greenlip abalone (135 to 185-mm length) were collected from several locations in northern Tasmania, 40° to 41°50'S, 146°50' to 148°50'E (Petal Point, Foster Islands, Port Sorell, and Flinders Island) and maintained in recirculating systems. Macroalgae were collected in southern Tasmania, 42°50' to 43°50'S, 147°50' to 148°E (Blackman Bay and Port Arthur). Macroalgae of the genera *Polysiphonia* sp., *Ulva* sp., and other epiphytic algae associated with the macroalgae *Amphibolus* spp. collected by divers were used as food for the abalone (Harris et al. 1998). The algae were added to the maintenance tank and left for 10 to 14 days. To remove algal debris, tanks were siphoned every second day. A diatom film, which was grazed by the abalone, developed within the tank during the study period.

Preparation of Abalone for Experiments

Abalone were removed from the maintenance tank with either a commercial abalone iron, a warm water siphon, or a flat plastic spatula with grease. Abalone were anesthetized in 1 mL/L of ethyl p-aminobenzoic acid (benzocaine) solution for 15 minutes to prevent any movement. The stock benzocaine solution was made up from 100 g ethyl p-aminobenzoic acid dissolved in 1 L of 95% alcohol (Hahn 1989).

Microprobe Analysis of the Abalone Digestive Tract Microenvironment

Seven adult abalone, 145 to 160-mm, were used for pH analysis. Two of these were also used for determining dissolved oxygen levels in the gut. Once anesthetized, the abalone were removed from their shells, and the integument was removed to expose the gut (see Harris et al. 1998). All measurements were recorded after the electrodes were fully inserted into the lumen and the electrode response stabilized, which took less than 4 seconds.

The pH microelectrode was a MI-413 microcombination pH electrode in an 18-gauge needle (Microelectrodes Inc., New Hampshire, USA). It was connected to a Hanna Instruments (HI 9017) microprocessor pH meter, accurate to ± 0.0001 mA. The pH electrode was calibrated with buffered solutions of pH 7.00 and pH 4.00, at 20°C. pH measurements were performed at 18 to 20°C and 35.5 ppt.

The dissolved oxygen probe was a Clark-type oxygen microelectrode (OME) with guard cathode (Diamond General Corp., MI, USA). It was used with a Keithley 485 autoranging picoammeter. The dissolved oxygen electrode was prepared for calibration by immersion in air-saturated water for 30 min. The current output was measured for 1 hour after saturating aquarium water of 35.5 ppt and held at $16.1 \pm 0.05^\circ\text{C}$ with N_2 , air or O_2 . The electrode had good linearity in its response and a low stirring effect of 1.2%, which was considered negligible (Revsbech and Jørgensen 1986). Before testing each animal, water samples were taken for Winkler analysis to check electrode calibration. Electrodes were held in position using a micromanipulator (Maerzhauser, Germany), attached to a heavy stand (Fig. 1). Any lateral stress on an OME may cause it to crack, so the abalone were held in position by pinning them to a soft polystyrene base. The base was fixed to a wire rack and positioned within a small (30 × 30 × 20 cm) aquarium con-

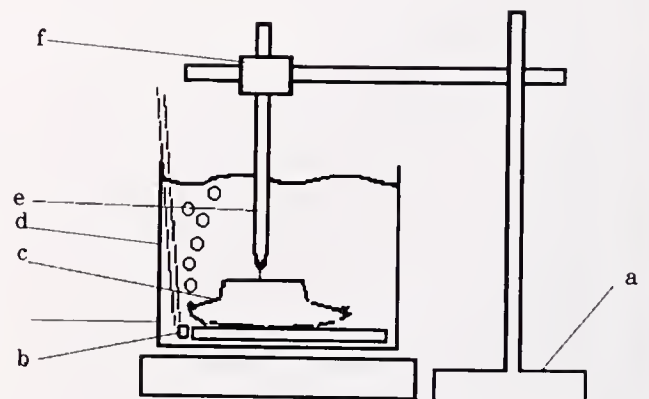


Figure 1. Experimental setup (a = 15 kg stand base; b = airline; c = abalone; d = seawater aquarium; e = dissolved oxygen OME; f = micromanipulator).

taining seawater. The gut wall was punctured with the electrode, which was then inserted into the lumen. Positioning the OME in the lumen was achieved once the gut wall slid up the probe.

OME's give an output in pA which is directly related to the partial pressure of oxygen. Because temperature and salinity were constant, the dissolved oxygen concentration was calculated as:

$$\text{DO sample} = \frac{\text{I sample}}{\text{I standard}} \times \text{DO standard}$$

(I sample = current output in pA; I standard = current output from aquarium water; DO standard = DO of aquarium water as measured by Winkler titration; DO sample = DO of sample in mg/L.)

The limit for detection by the OME was 15 pA, or 0.38 mg DO.L⁻¹. The oxygen saturation can be calculated by dividing the calculated concentration in mg/L by the saturated concentration of oxygen at 16.1°C and 35.5 ppt, which is 7.93 mg/L.

Bacterial Flora of the Abalone Digestive Tract

To obtain bacterial samples from the abalone, sections of the digestive tract (postesophagus region I, crop, stomach, style sac, and intestines) were excised with scissors, scalpel, and tweezers (Harris et al. 1998) and placed into each of three enrichment broths (CarboxyMethylCellulose (CMC), starch, or agar). Smaller sections, such as the esophagus, were sampled as whole gut sections; whereas, larger areas, such as the stomach, required the removal of one wall. Before use, these broths were boiled for up to 30 minutes to remove dissolved oxygen, then placed in an ice-bath to cool rapidly. Enrichments were performed in anaerobic and microaerophilic conditions according to methods modified from Lewis et al. (1992). Carbohydrate enrichment broths were incubated at 21°C and examined on the third day.

Loops of enrichment broth were transferred to solid media containing either starch, agar, or CMC. CMC and starch plates were made using modified seawater, agar, and vitamins (SWAV) medium with agar at 10 g/L and CMC or starch at 10 g/L. pH concentrations of enrichments broths and solid media were adjusted to 6.5 for esophagus and intestines II and IV, and 5.5 for crop, stomach, and style sac samples. Plates were incubated in anaerobic and microaerophilic conditions at 21°C and examined after 4 days. Visible colonies were subcultured onto the same media to purify the isolates. Bacteriological peptone and yeast extract were omitted from the (SWAV) medium of Lewis et al. (1992) to enable single carbon sources (starch, agar, or CMC) to be added.

CMC was dissolved in distilled water before adding to seawater. CMC degradation was detected visually by observing clearance zones around colonies. Localized clearance of the medium was taken as hydrolysis. Hydrolysis on agar plates was seen by a lowering of colonies into the agar. Combined ingredients for solid media were autoclaved at 121°C and 15 psi for 15 minutes.

Tests performed on pure isolates were: gram reaction and cellular morphology, colonial morphology, OF test, Craigie tube motility test, catalase, oxidase, and susceptibility to the antibiotic 0/129 (150 µg) (Oxoid). Organisms found to be fermentative were tested against this antibiotic for presumptive *Vibrio* spp. identification. All three media types were tested for their effect on the catalase and oxidase reactions and were found to have no effect.

Statistical Analysis

Data were subjected to single, fixed factor analysis of variance (ANOVA) after meeting assumptions of normality using the Shapiro-Wilk test (Zar 1996) and homogeneity of variance using Cochran's test (Underwood 1981). Multiple comparison of means (Tukey-Kramer HSD) was performed on data that showed a significant ANOVA result (Sokal and Rohlf 1995). All analyses were conducted using JMP 3.0 software (SAS Institute).

RESULTS

Microenvironment of the Abalone Digestive Tract

Readings below the limit of detection for the OME (0.38 mg DO.L⁻¹) indicated that conditions were, at least, microaerophilic. This was consistently found throughout the length of the digestive tract, with little variation evident. All the dissolved oxygen concentrations were calculated to be ≤5.7% oxygen saturation at 16.1°C and 35.5 ppt. The crop was significantly ($p < .05$) more acidic than the esophagus and the intestines (Table 2).

Bacterial Flora of the Abalone Digestive Tract

The attempt at isolating anaerobic bacteria produced few organisms. The microaerophilically incubated plates showed growth after 4 days incubation and were subcultured after 7 days. Approximately three bacterial types were evident on each plate for a total of 51 isolates.

Subculturing revealed both pigmented and nonpigmented colonies, mostly as Gram-negative rods. Strains showed both negative and positive catalase reactions, and all strains examined were oxidase negative. Physiological types included mostly fermentative reactions (36 isolates), although no reaction (13 isolates), and oxidative reactions (two isolates) were also observed. Only two isolates were obligate microaerophiles, both being members of the family Enterobacteriaceae; the remainder were facultatively aerobic. Most fermentative strains were resistant to 0/129, although six isolates showed inhibition zones ranging up to 20 mm.

Most of the microbial isolates showing positive hydrolytic activity occurred within the oesophagus (11 isolates) and intestines (17 isolates). Fewer isolates were recovered from the crop, stomach, and style sac. Hydrolytic activity varied among the isolates (Table 3), being prevalent among those isolates identified as *Cytophaga* spp. and Enterobacteriaceae. Degradative ability on agar and CMC was common among the isolates, although no isolates were observed that could degrade both starch and agar.

TABLE 2.

pH profile of the digestive tract of greenlip abalone, *H. laevigata*.

Sampling Site	Mean ± SE	n
Esophagus	6.20 ± 0.16 ^a	8
Crop	5.28 ± 0.08 ^b	8
Stomach	5.53 ± 0.10 ^{a,b}	8
Style sac	5.49 ± 0.12 ^{a,b}	7
Intestine II	5.80 ± 0.12 ^a	8
Intestine III	6.34 ± 0.04 ^d	7
Intestine IV	6.65 ± 0.06 ^d	8
Intestine V	6.64 ± 0.04 ^d	3

Means sharing a common superscript are not significantly different ($p > .05$).

TABLE 3.

Genera, location, and hydrolytic activity of bacterial isolates from the digestive tract of *H. laevigata*.

Site	Bacterial Groups	No. Isolates	Polymer Degrading Activity ^a		
			CMC	Agar	Starch
Esophagus	Enterobacteriaceae	6	4	3	2
	<i>Cytophaga</i>	4	4	4	
	<i>Alteromonas</i>	1	1	1	
Crop	Enterobacteriaceae	6	4	3	
	<i>Aerococcus</i>	2	2		
Stomach	Enterobacteriaceae	6	3	1	3
	Neisseriaceae	1	1		1
Style sac	Enterobacteriaceae	6	4	1	2
	Neisseriaceae	1			
	<i>Alteromonas</i>	1			
Intestine II	Enterobacteriaceae	6	5	1	3
	<i>Alteromonas</i>	1		1	
	<i>Listeria</i>	1			1
Intestine IV	Enterobacteriaceae	4	2	1	
	<i>Cytophaga</i>	3	3	3	
	<i>Acinetobacter</i>	1	1		1
	<i>Aerococcus</i>	1			
Total		51	34	19	13

^a Number of isolates showing positive hydrolytic activity.

DISCUSSION

Within the abalone gut, dissolved oxygen levels were below the detection level of this OME and the gut should, therefore, be regarded as anoxic, or at least microaerophilic. Low dissolved oxygen levels, similar to those found within the abalone digestive tract, have been reported in other invertebrates. Plante and Jumars (1992) found that even within the digestive tracts of deposit-feeders known to have consumed oxygenated sediment, oxygen levels were similar to animals known to have consumed anoxic sediments. From this, Plante and Jumars (1992) proposed that the low dissolved oxygen levels were attributable to biological or chemical processes in the foregut that quickly consumed added oxygen, with the gut contents effectively acting as an oxygen sink.

The low oxygen tension and weakly acidic conditions within the digestive tract of *H. laevigata* provide a selective environment. Prieur et al. (1990) reviewed the microbiology of bivalve digestive tracts and noted a higher proportion of fermentative bacteria than in the surrounding seawater. Most of the bacteria isolated from the guts of aquatic invertebrates have been facultative aerobes, although obligate aerobes and anaerobes have been reported (Harris 1993). The metabolism of facultative aerobes quickly depletes the available oxygen, thereby creating conditions favorable for anaerobic fermentation. However, anoxia is an insufficient variable with which to define microbial activity, and fermentation in particular, in an environment such as the abalone gut, because an anoxic environment may still have oxidizing conditions. Combined Eh and dissolved oxygen measurements provide a better understanding of the microbial environment (Plante and Jumars 1992).

The pH profile along the greenlip abalone digestive tract is similar to other gastropod and bivalve mollusks. The lowest values recorded are in the stomach of *Patella* sp. (5.55) (Hyman 1967), *Crepidula* sp. (6.00) (Hyman 1967), *Buccinum* sp. (5.6) (Hyman

1967), *Ostrea edulis* sp. (6.02) (Mathers 1974), and in the style sac of *Mya* sp. (4.4) (Owen 1966), although few authors have reported pH levels in the crop of mollusks. The lower pH in the crop and stomach reduces the viscosity of mucus, allowing the gut contents to mix readily. Raising pH increases the viscosity of the mucus in the intestine, helping to consolidate the loosely bound mucus string into cohesive pellets (Morton 1968). Crop contents in *H. cracherodii* are considerably less viscid than in other regions of the gut (Campbell 1965). The only direct measurement of pH within the digestive tract of abalone was described by Gómez-Pinchetti and García-Reina (1993). They measured the pH of digestive gland homogenates from *Haliotis coccinea canariensis*, and recorded values between 5.5–6.0. This suggests that the crop is the most acidic region in haliotids in general and specifically in *H. laevigata*. This organ is believed to act as a food storage and digestion organ, because both recognizable food pieces up to 3-cm long and unrecognizable food have been found (Campbell 1965). Esophageal valves restrict the movement of larger food particles from the crop into either the stomach or the stomach cecum (Crofts 1929).

Natural seawater has pH values varying between 7.5 to 8.5 (Austin 1988). The decrease in pH within the abalone gut portrays an environment that differs from relatively stable, alkaline seawater. The acidic abalone gut provides an environment that would select against organisms unable to tolerate acid pH. The genus *Vibrio*, for example, is tolerant of mildly alkaline conditions and is generally grown on media of pH 8.6 (Baumann and Schubert 1984); whereas, other marine bacteria such as *Alcaligenes* are commonly isolated in neutral pH (Kerstens and De Ley 1984).

The enzyme activity peaks found in other abalone also illustrate the pH changes found throughout the gut of *H. laevigata*. Digestive activity in the esophagus of *H. rufescens* is highest at pH levels between that of seawater and 6.8 (McLean 1970). Peak alginase activity in *H. rufescens* and *H. corrugata* occurs from pH 7.4 to 7.6 (Nakada and Sweeney 1967). However, the lower pH levels found in the crop of *H. laevigata* are still within the range of pH that enables efficient amylase and protease activity in *H. rufescens* (McLean 1970), even though different enzymes with different pH optima are likely to be present in *H. laevigata*.

The stomach functions to collect food and secretions from the salivary glands, cecum, and the digestive glands (Crofts 1929), so the pH within the stomach should also be a mixture of these influences and the secretions of the stomach. Observations from *H. laevigata* indicate that the stomach has similar pH to that of the crop and style sac. In the digestive diverticula of *Haliotis* sp. (= *Haliotis*), the maximum activity of enzymes such as fucoidanase occurs at pH = 5.4 (Thanassi and Nakada 1967), a value of pH similar to that found in the crop, stomach, and style sac of *H. laevigata*.

In some invertebrates, pH and redox conditions are sometimes at unusual levels that favor association between the host and specific microbial communities (Harris 1993). The selective nature of these changes in pH imparted on the microbial communities will favor those microbes best adapted to the conditions. The different pH readings and the microaerophilic environment found in the abalone digestive tract, therefore, provide several niches for microbes to exploit and grow.

Most of the bacteria found within the abalone gut were able to degrade starch, CMC, or agar. The ability of several different isolates to degrade both agar and CMC indicates that these bacteria were capable of growth on two of the more common substrates available in this environment. Although the isolation media were

as close as possible to the conditions within the gut environment, the use of selective media can sometimes fail to detect some bacteria capable of hydrolytic activity (Harris 1993). Therefore, it is likely that there are some bacterial types present that were not isolated through the enrichment process. The larger diversity of bacteria isolated from the esophagus of the abalone, with a decrease in species in the crop and stomach, is directly related to the selective conditions of the gut environment. Dissolved oxygen and pH are two variables likely to influence microbial growth strongly in the abalone.

Bacterial genera found within the digestive tracts of bivalve molluscs include: *Achromobacter*, *Flavobacterium/Cytophaga*, *Pseudomonas*, *Vibrio*, *Corynebacterium*, *Arthrobacter*, *Escherichia*, *Neisseria*, *Streptococcus*, *Micrococcus*, *Moraxella*, *Acinetobacter*, and *Aeromonas* spp. (Prieur et al. 1990). Juvenile blacklip abalone, *Haliotis rubra*, have been shown to consume bacteria with coralline algae (Garland et al. 1985). These bacteria were predominantly *Moraxella*, although *Pseudomonas*, *Vibrio*, *Alteromonas*, and smaller numbers of *Flavobacterium/Cytophaga* and *Aeromonas* spp. were also present. Bacterial isolates obtained from the South African abalone, *H. midae*, showed an ability to use a range of complex polysaccharides (Erasmus et al. 1997). In terms of hydrolytic capabilities, the types of bacteria found within the abalone gut are similar to those found in the sea hare (Gastropoda), *Aplysia juliana*, (Vitalis et al. 1988). However, not all the bacteria ingested by abalone may be able to exploit the gut environment. From our study, it seems that the marine bacteria capable of growth at reduced pH are different in composition to those isolated by other authors at higher pH (Sawabe et al. 1995, Erasmus et al. 1997). Consequently, the reports of other authors may have revealed bacterial populations that are present, but not necessarily capable of contributing to the digestive ability of the host in a typical gut pH regime. It may be that the bacteria reported in this study differ from those reported elsewhere by being capable of digesting algae within the gut environment, from the wider variety of bacteria ingested by the abalone.

Vibrio spp. have been recorded as predominant microorganisms in several marine invertebrate digestive tracts (Unkles 1977, Sochard et al. 1979, Harris 1993), including abalone (Erasmus et al. 1997, Sawabe et al. 1995). It may seem surprising that so few isolates of *Vibrio* spp. were obtained from the greenlip abalone. However, *Vibrio* spp. are usually isolated on alkaline media (Baumann and Schubert 1984), suggesting growth is reduced or prevented in acidic conditions. The microbial isolates from the most acidic region, the crop, were almost entirely from the family Enterobacteriaceae, suggesting that these bacteria are well adapted to the acidic gut environment. The Enterobacteriaceae are rarely recorded from the marine environment or from the guts of invertebrates (Harris 1993). The occurrence of the Enterobacteriaceae in *H. laevisgata* may represent a normal bacterial flora that specifically developed within the gut and adapted to the reduced pH and microaerophilic environment. Their presence throughout the digestive tract suggests that these bacteria may be indigenous.

Wild greenlip abalone are obligate drift algae consumers, able to consume many different types of algae (Shepherd 1973). Ingestion of diatoms, detritus, bacteria, and sand also occurs as a result of the mode of feeding (Campbell 1965). The diet fed to the abalone during this study was limited in diversity as compared to that of abalone in the wild. The restrictions this would place on microbial growth may be subtle, although some decrease in normal microbial species diversity could be expected. By restricting avail-

able food types, this may also reduce bacterial diversity. Digestive tract analysis of bacterial biota in other animals maintained in laboratory systems supports this theory, because the selective pressures imposed by the artificial environment influence the normal bacterial flora occurring in the gut of aquatic invertebrates (Sochard et al. 1979).

Seaweeds are known to have epiphytic colonies of diatoms, yeasts, and bacteria (Austin 1988), some of which have known algal cell-degrading abilities, such as *Cytophaga* spp. Mechanical breakdown of algae by the radula would release cellular contents previously unavailable to epiphytic or free-living bacteria, and this would be expected to stimulate microbial growth. However, few bacteria were seen to be associated with the gut surface of the greenlip abalone. This may be caused by the action of cilia and the presence of mucous and secretory cells (Harris et al. 1998), and the results from this study that suggest that variation in pH from the external environment may also be a factor. We obtained 44 isolates of bacteria from the gut of *H. laevisgata*. These bacteria were capable of degrading algal polysaccharides at levels of pH and dissolved oxygen similar to gut values. Therefore, bacteria may contribute to *H. laevisgata* nutrition. Because the bacteria do not seem to be strongly associated with the gut epithelium, then bacterial digestive activity is likely to be restricted to the lumen (Harris et al. 1998). Some output of feces still occurs several days after feeding has ceased (Wee et al. 1992), allowing the possibility for sustained bacterial activity within the intestines. The less intimate association of bacteria with bivalves as compared to terrestrial animals (Kueh and Chan 1985) also seems apparent in the greenlip abalone. Kueh and Chan (1985) suggested that, for oysters, the gut flora are mainly derived from the external environment and a more indigenous population of bacteria dominate the lower digestive tract because of selective pressures and multiplication. This situation seems analogous to that of the greenlip abalone.

CONCLUSION

The presence of bacteria within the digestive tract of the greenlip abalone, and their ability to break down algal carbohydrates at pH levels found within the gut, suggests that bacteria are capable of contributing to the nutrition of their host, although the amount remains in question. The lack of physical association of these bacteria with gut epithelium suggests a different digestive strategy to terrestrial herbivores. If bacteria contribute significantly to host nutrition, they are more likely to contribute through activity within the gut lumen. The selective pressures of the gut environment give rise to bacterial populations that are different in composition to those reported from the external marine environment.

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BACTERIAL COLONIZATION OF A FORMULATED ABALONE DIET DURING EXTENDED IMMERSION

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ABSTRACT The characteristics of the microbiota of a formulated abalone (*Haliotis laevis*) diet were studied by scanning electron microscopy (SEM) and standard bacterial culture and taxonomic techniques. Microbes colonizing the diet (ABCHOW) were enumerated by SEM and partly identified after immersion of the diet in seawater for 0, 2, and 4 days with and without abalone. The fatty acid composition of the diet was also analyzed, after similar treatments, for biomass estimates and bacterial biomarker identification. Bacterial numbers on unimmersed diet and diet immersed in sterile seawater for 2 and 4 days were negligible. Bacteria proliferated after 2 days immersion in seawater with abalone (1.2×10^5 cells/mm²) and without abalone (5.7×10^4 cells/mm²) ($p < .05$). Numbers continued to rise between 2 and 4 days for diet immersed without abalone (6.5×10^4 cells/mm²). However, a decrease in bacterial numbers was observed between 2 and 4 days immersion in seawater with abalone (7.7×10^4 cells/mm² after 4 days), and this was accompanied by an increase in ciliate numbers (from 0 to 10^2 ciliates/mm²). Ten distinct taxonomic groups of bacteria were identified from the diet after immersion; *Cytophaga* spp. was the most abundant group. Chemotaxonomic analysis, including fatty acid profiling, failed to provide microbial biomass estimates or bacterial biomarkers. The majority of the microbes were found to have the capacity to degrade a protein and a lipid source within the diet, but not two carbohydrate sources, including the binder. Bacteria were found to affect the physical form of the diet, but it is unlikely that they affected its macronutritional value to any great extent.

INTRODUCTION

Worldwide decline in abalone fisheries has accelerated the development of abalone mariculture (Coote et al. 1996). One of the major constraints to the industry is the provision of an economically viable, nutritionally suitable, formulated diet (Fleming et al. 1996). Given the high cost and logistical difficulties of supplying natural seaweeds to abalone (Coote et al. 1996), the industry preference is for a cost-effective formulated diet. The development of such a diet depends not only on an understanding of the nutritional requirements of the abalone and their digestive processes, but also on the diet's performance in the culture system.

Most formulated diets for fish are consumed rapidly, but, large industries now exist for slow-feeding invertebrates such as marine shrimp and marine gastropods. Abalone graze on food slowly, so it is not uncommon in commercial situations for diet to be immersed for up to 4 days before it is consumed. Given the rapid leaching of water-soluble nutrients from formulated diets (Goldblatt et al. 1979) and the ample time for microbial colonization, costly feeding strategies, based on frequent input, may be adopted. However, growth trials have suggested the opposite may actually be the case: faster growth rates were observed with less frequent feeding and cleaning intervals and therefore extended immersion (Maguire et al. 1996).

When considering the effects of microbes on the nutritive value of formulated diets, several possibilities exist. They may have detrimental effects: by consuming the nutrients meant for the abalone, or decreasing the stability of the diet by facilitating its physical breakdown and exacerbating water quality problems (Shigueno

1975, Moriarty 1986). The diet may also act as a reservoir for pathogenic microbes (Moriarty 1986, Muir and Sutton 1994). Conversely, bacteria may be beneficial by forming extracellular particulate matter after uptake of dissolved nutrients (Pearl 1978), which may then become available to the abalone. Dietary constituents may be broken down and consumed by bacteria, which may in turn be consumed by the abalone. Both Garland et al. (1985) and Harris (1993) state that digestive enzymes may be supplied to abalone by bacteria. Finally, bacteria may produce certain feed attractants or micronutrients that make the diet more palatable or nutritionally adequate (Sakata 1987, McShane et al. 1994).

The aim of this study was to quantify the microbial population colonizing a formulated abalone diet after periods of extended immersion and to isolate and partially identify members of this microbial population in order to examine their exoenzyme activity in relation to diet constituents. This information was sought using three distinct methods: scanning electron microscopy (SEM) enumeration, traditional biochemical methods, and chemotaxonomic techniques. Results from this study could allow more informed decisions regarding feed strategies and diet formulation.

MATERIALS AND METHODS

Diet

The formulated diet was a proprietary formulation (ABCHOW) produced with a pasta maker and subsequently dried as a biscuit ($14 \times 9 \times 1$ mm). ABCHOW was supplied by the South Australian Research and Development Institute (SARDI), Adelaide, Australia and was derived from diet 9 as used by Fleming et al. (1996). Diet was stored in a domestic freezer and added to treatment tanks manually.

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Abalone Culture Systems

Treatment tanks comprised 70-L, round, aerated, center-draining, flow-through, fiberglass aquaria supplied with approximately 1.5 L/min of sand-filtered (40 to 50 μm) sea water. Each treatment utilized three replicate tanks. Tanks were continuously shaded in a black plastic (100% shade) enclosure to prevent excessive benthic diatom colonization. The entire system was housed within a translucent fiberglass building. Light intensity was < 0.03 microEinsteins/ m^2 . Treatment tanks using abalone contained a biomass of approximately 100×1 g abalone/tank. Trials were conducted at ambient conditions (typically about 14°C, pH 8.2 and salinity 34 to 35 ppt) at a commercial abalone farm, Marine Shellfish Hatcheries P/L, Bicheno, Tasmania, Australia. Tanks were cleaned by rapid draining before the sample diet was added.

Treatments

Seven treatments were employed in this study:

1. Two days immersion in the presence of abalone
2. Two days immersion, abalone absent
3. Four days immersion in the presence of abalone
4. Four days immersion, abalone absent
5. Zero days immersion
6. Two days immersion in sterile seawater
7. Four days immersion in sterile seawater

For treatments 1 to 4, samples were collected for both SEM enumeration and bacterial isolation directly from the aquaria. For treatments 6 and 7, 200 mL of coarse-filtered, aged seawater was added to 500 mL glass beakers and autoclaved for 15 min at 121°C, 15 psi. Diet was added aseptically, and the beakers were then incubated under similar conditions to those experienced by other treatments, except they were static.

Bacterial Isolation and Identification

For isolation of bacteria, five pieces of the feed were collected from each replicate tank or beaker. Samples were collected using methylated-spirit disinfected filter forceps and placed in 10 mL of sterile saline. They were then hand homogenized and a further four, tenfold serial dilutions performed. Samples from the five dilutions were subsequently inoculated onto general carbon source plates and restricted carbon source plates and incubated at room temperature for 1 to 14 days. Restricted carbon source plates were used in the study to isolate bacteria utilizing nutrients specific to the ABCHOW diet. The restricted carbon source plates comprised 20 g agar, 10 g carbon source, and 500 mL each of distilled and filtered seawater. The nutrient source for each plate consisted of one of the principal ingredients of the ABCHOW diet: two carbohydrates (semolina), including the binder (sodium alginate), one high protein meal (casein), and one lipid (fish oil) source. Following incubation, plates were inspected daily for growth. Discrete colonies were removed and transferred to separate abalone-feed-nutrient-agar plates (identical to those above, substituting homogenized ABCHOW diet for the individual nutrient sources) for purification. Subculturing continued until pure cultures were obtained, and these were maintained on Ordal's medium (Atlas 1993).

Identification tests performed on the pure cultures were: Gram reaction, cellular and colonial morphology on Ordal's medium after 5 days incubation; glucose utilization (OF) test for metabolic type using a modification of the method of Barrow and Feltham (1993), whereby 500 mL of filtered seawater was substituted for

the equivalent amount of distilled water; Craigie tube motility test (similarly modified from Barrow and Feltham 1993); oxidase and catalase (Barrow and Feltham 1993); anaerobic growth (Oxoid, Anaerogen system) on Ordal's medium; sensitivity to the antibiotic O/129 (Oxoid), and growth on the restricted carbon sources specific to the ABCHOW diet.

SEM Enumeration

For each replicate tank or beaker, three whole pieces of feed were used. These were fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.4) containing the major marine salts (Garland et al. 1982) immediately upon removal from experimental tanks. Samples were thus fixed for 2 h at room temperature, rinsed for 20 min in 0.1 M cacodylate buffer (Hodson and Burke 1994) and dehydrated through a graded ethanol (EtOH) series (Hodson and Burke 1994). The dehydration series was suspended at 70% EtOH until critical point drying was possible.

Dehydrated samples were transferred to acetone and critical point dried using liquid CO_2 . Samples were dried using a Baltec CPD 030 or a Polaron E3000 CPD. Following drying, samples were mounted onto aluminum SEM stubs with conductive carbon paint as the adhesive. Samples were then sputter coated with gold (Balzers type coater) twice, to improve sample stability (Rosowski et al. 1981), as soon as practicable after drying. Samples were then stored in a vacuum desiccator (25–30 psi) over CaCl_2 (Garland et al. 1982). Samples were viewed and photographed under a Philips 505 SEM at a voltage of 15 kV.

Random fields on the sample surface were chosen at low magnification (Lewis et al. 1985), then examined at a magnification of 2500 X, giving a viewing field of 1322 μm^2 (although it should be noted that the viewing field was not flat). The surface was focused and aligned at 90° to the viewing plane, and all organisms within the viewing field (including those that intersected the top and left sides) were counted. For each replicate, at least 10 full fields were counted. Representative micrographs were taken of each replicate.

Data were transformed with a square root transformation (Sokal and Rohlf 1987) prior to statistical analysis, with a one-way analysis of variance (ANOVA), to meet assumptions of normality and homogeneity of variance. For all tests, a significance level of $p < .05$ was adopted. Data for each immersion period were analyzed separately, and environment (abalone present or absent or sterile seawater) was considered as a fixed factor. Pairs of means were compared using Fishers LSD (Sokal and Rohlf 1987).

Fatty Acid Analysis

The method of Bligh and Dyer (1959), as modified by Dunstan et al. (1995), was used for extraction. Fatty acid methyl ester (FAME) samples were analyzed with a Hewlett-Packard 5890 gas chromatograph (GC) that was equipped with a flame ionisation detector (FID). FAME samples were injected using an air-cooled on-column injection into a polar BPX-70 fused silica column (50 m \times 0.32 mm ID). High-purity H_2 was the carrier gas. The GC oven temperature was initially held at 45°C for 2 min after injection and then increased at 30°C/min to 120°C and at 3°C/min to 240°C, and was then held constant for 10 min. The retention index on both polar and nonpolar columns was used to identify fatty acids. Fatty acid identifications were verified with a Hewlett-Packard 5970B GC/MS system.

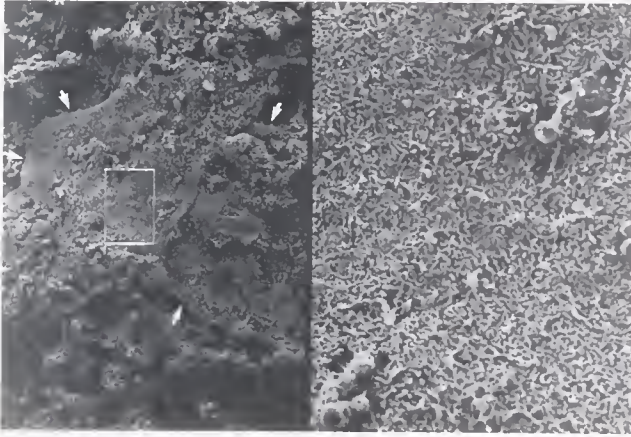


Figure 1. A split view of the surface of diet immersed for 4 days without abalone. The left of the micrograph shows the interface between areas of confluent growth and very little growth (arrows indicate boundary). The right demonstrates the density of cells within the area of growth. (Bar = 0.5 mm and refers to the left of the micrograph).

RESULTS

Enumeration

At low magnification, the surface of each sample was observed to be typically undulating with irregular depressions. The irregularity of the diet's topography was accentuated with immersion time. At high magnification (2,500 X) it was possible to distinguish individual bacterial cells, despite the surface corrugations and often dense mucilage. Cells colonized different areas of the diet to differing degrees; lipid globules (identified visually under EM) were less densely colonized than the rest of the diet matrix (Fig. 6). Areas of confluent growth, adjacent to areas of no growth, were also evident (Fig. 1). These areas of confluent growth seemed

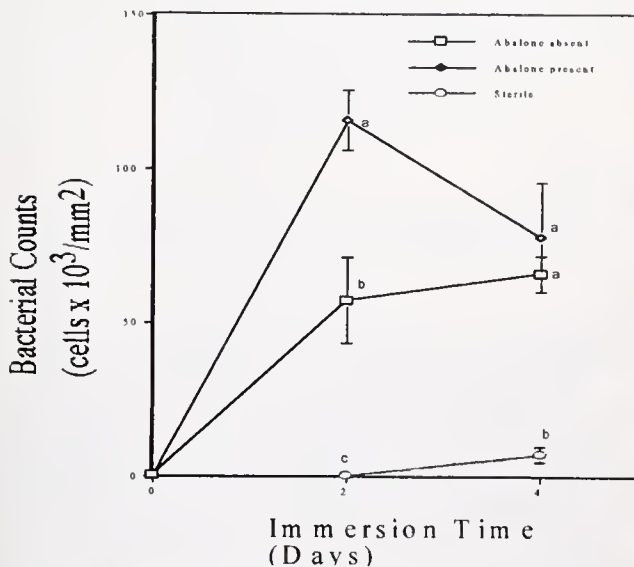


Figure 2. Mean bacterial numbers on ABCHOW diet at 0, 2, and 4 days immersion with abalone, without abalone, and in sterile seawater. Means for the same immersion time that share a common superscript are not significantly different ($p > .05$). Vertical lines represent standard errors of the means ($n = 3$).

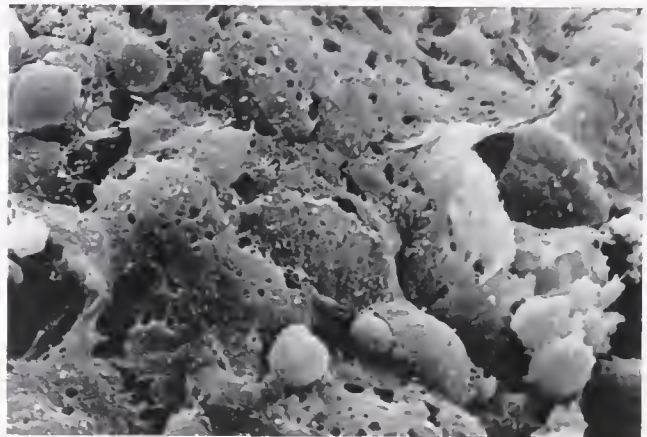


Figure 3. A typical random counting area from unimmersed diet (2500 X, Bar = 10 μ m). No bacterial cells were present.

to be the result of bacteria spreading from initial sites that were favorable for growth. SEM examination showed negligible bacteria colonizing treatments 5 to 7, but a substantial proliferation in bacterial numbers for treatments 1 to 4 (Figs. 2–4).

One-way ANOVAs, based on all treatments except 5, confirmed a significant treatment effect ($p < .001$) on bacterial abundance for 2- and 4-day data. Comparisons of means for the same immersion time showed that bacterial numbers for all treatment levels ($n = 3$) (abalone absent = 5.7×10^4 cells/mm², abalone present = 1.2×10^5 cells/mm², sterile = 1.5×10^2 cells/mm²) were significantly different after 2 days immersion ($p < .05$). There was no significant difference between the bacterial densities for nonsterile treatments after 4 days, with (mean = 7.7×10^4 cells/mm²) or without (mean = 6.5×10^4 cells/mm²) abalone, but mean counts from diet immersed in sterile seawater (mean = 7.0×10^3 cells/mm²) were significantly different ($p < .05$) to other treatments after this immersion time.

SEM also revealed the presence of ciliates (Fig. 5) on the diet subjected to treatment 3. The average number of ciliates present on treatment 4 diet was 0.8 ciliates/counting field. The ciliates were approximately $30 \times 20 \mu$ m in size, were all of the same morphotype and were observed on all replicate samples from treatment 3, but no other samples.

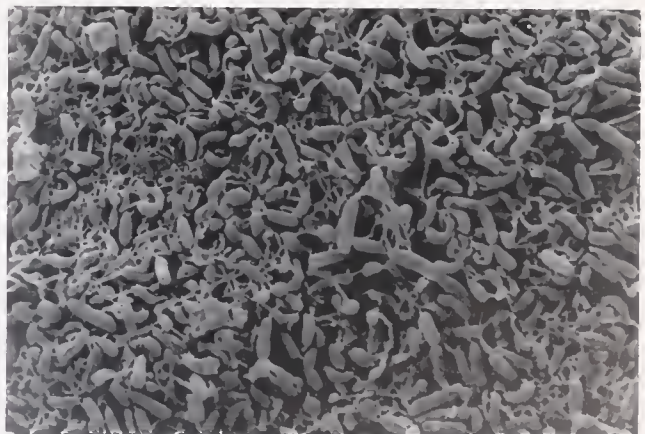


Figure 4. A typical random counting area from diet immersed for 4 days, without abalone, (2500 X, Bar = 10 μ m) showing massive bacterial colonization.



Figure 5. A micrograph of an unidentified ciliate (193000 X, bar = 10 μ m), typically observed on treatment 4.

Identification

A large number of bacteria (108) were isolated directly from agars containing ingredients of the ABCHOW diet. No bacteria were isolated from diet immersed in sterile seawater, because no colonies had formed on plates from these treatments after 14 days. Bacteria were identified and placed into one of 10 taxonomic groups (Table 1). Most groups were observed in all treatments; bacteria falling into the group *Cytophaga* being the most numerous.

The identification scheme used was adopted from Cropp and Garland (1988). All isolates were Gram-negative rods, catalase (+) and grew aerobically on Ordal's medium. Isolates were initially separated on their ability to produce acid from glucose (OF test) and on their ability to grow anaerobically on Ordal's medium. Three distinct groups resulted (Table 2). The first group were able to produce acid from glucose aerobically, but were incapable of anaerobic growth on Ordal's medium. The second group did not produce acid from glucose aerobically or anaerobically; hence they were oxidative, but some were capable of anaerobic growth on Ordal's medium. The final group were fermentative, producing acid from glucose both aerobically and anaerobically and were capable of anaerobic growth on Ordal's medium.

All bacteria isolated demonstrated the ability to degrade a wide range of carbon sources (Table 3). Results were similar for both



Figure 6. Lipid globules in the diet from 2 days immersion, with abalone (2500 X, Bar = 10 μ m), showing typical sparse colonization of the lipids.

carbohydrates. All isolates were able to use protein meal as a nutrient source. At least one isolate from each treatment was able to degrade either the carbohydrate or the lipid sources. At least one isolate from each taxonomic group in treatment 1 was able to utilize all nutrient sources. However, members of the families Vibrionaceae and Enterobacteriaceae demonstrated poor utilization of the binder. No *Pseudomonas* or *Acinetobacter* spp. was observed to utilize the carbohydrate source. All other groups determined to have oxidative metabolism were generally poor users of carbohydrate energy sources. Lipase activity was expressed by a large proportion of isolates of most taxonomic groups.

Fatty Acid Analysis

Analysis of the ABCHOW diet's fatty acid content after 0, 2, and 4 days immersion (with and without abalone) showed an increase in total fatty acids from 3.26 g/100 g dry wt at 0 day immersion, to 4.55 g/100g dry wt at 4 days immersion without abalone (Table 4). Analysis of straight and branched-chain fatty acids in the ABCHOW diet revealed no change in relative composition after extended immersion (Table 5, 6).

DISCUSSION

Enumeration

Microorganisms are known to colonize many natural and artificial marine surfaces, hence it is not surprising that this study

TABLE 1.

Bacterial types isolated and identified from treatments 1 to 5, showing the total numbers of isolates in each taxonomic group.

Bacterial Type	Number of Isolates	Treatments
<i>Cytophaga</i>	27	1 to 5
<i>Mesophilobacter</i> or <i>Moraxella</i>	19	1 to 5
Vibrionaceae	17	1 to 5
<i>Alcaligenes</i> or <i>Pseudomonas</i>	12	1 to 5
Enterobacteriaceae	13	2 to 5
<i>Acinetobacter</i>	8	2 to 5
<i>Moraxella</i> or <i>Paracoccus</i>	5	1,2,3,5
<i>Aeromonas</i>	4	1,2,3
<i>Flavobacterium</i> or <i>Phenylobacterium</i>	2	3 & 4
<i>Alteromonas</i>	1	4
Total	108	

TABLE 2.

Metabolic types of bacteria isolated from treatments 1 to 5 as determined by glucose utilization (OF test) and anaerobic growth

Treatment	Oxidative Obligate Aerobe	Oxidative Facultatively Anaerobic	Fermentative	Total Number of Isolates
1		13 (9)	10	23
2		13 (2)	7	20
3		16 (2)	6	22
4	1	20 (3)	4	25
5		13 (2)	5	18
Total	1	75 (18)	32	108

Figures in parentheses indicate numbers of organisms capable of anaerobic growth.

TABLE 3.

Bacterial types isolated from treatments 1 to 5, showing total number of isolates in each group and the number of isolates capable of degrading specific nutrient sources found in the ABCHOW diet.

Treatment	Bacterial Group	Number of Isolates	Binder (Sodium Alginate)	Carbohydrate (Semolina)	Protein (Casein)	Lipid (Fish Oil)
1	<i>Aeromonas</i> spp.	2	1	1	2	2
1	<i>Alcaligenes</i> or <i>Pseudomonas</i> spp.	3	2	1	3	3
1	<i>Cytophaga</i> spp.	4	2	1	4	4
1	<i>Mesophilobacter</i> spp.	4	4	2	4	4
1	<i>Moraxella</i> or <i>Paracoccus</i> sp.	1	1	1	1	1
1	<i>Pseudomonas</i> sp.	1	1	1	1	1
1	<i>Vibrio</i> spp.	4	4	2	4	4
1	Vibrionaceae	4	0	1	4	4
2	<i>Acinetobacter</i> sp.	1	1	0	1	1
2	<i>Aeromonas</i> spp.	1	0	1	1	1
2	<i>Alcaligenes</i> or <i>Pseudomonas</i> sp.	1	0	0	1	1
2	<i>Cytophaga</i> spp.	7	1	4	7	7
2	Enterobacteriaceae	4	0	1	4	4
2	<i>Mesophilobacter</i> or <i>Moraxella</i> spp.	4	3	2	4	4
2	<i>Moraxella</i> or <i>Paracoccus</i> sp.	1	0	1	1	1
2	Vibrionaceae	3	0	3	3	3
3	<i>Acinetobacter</i> sp.	3	3	0	3	0
3	<i>Aeromonas</i> spp.	1	0	1	1	1
3	<i>Cytophaga</i> spp.	9	2	3	9	9
3	Enterobacteriaceae	2	0	0	1	2
3	<i>Flavobacterium</i> or <i>Phenylobacterium</i> sp.	1	1	0	1	1
3	<i>Mesophilobacter</i> or <i>Moraxella</i> spp.	1	1	0	1	1
3	<i>Moraxella</i> or <i>Paracoccus</i> sp.	1	0	0	1	0
3	<i>Pseudomonas</i> sp.	1	1	1	1	1
3	Vibrionaceae	3	0	1	3	3
4	<i>Acinetobacter</i> sp.	2	0	1	2	2
4	<i>Alcaligenes</i> or <i>Pseudomonas</i> spp.	4	0	0	3	3
4	<i>Aeromonas</i> sp.	1	0	1	1	1
4	<i>Cytophaga</i> spp.	4	1	1	4	4
4	Enterobacteriaceae	4	0	2	3	2
4	<i>Flavobacterium</i> or <i>Phenylobacterium</i> sp.	1	1	1	1	1
4	<i>Flavobacterium</i> sp.	1	1	1	1	1
4	<i>Mesophilobacter</i> or <i>Moraxella</i> spp.	7	1	1	6	6
4	<i>Pseudomonas</i> sp.	1	0	1	1	1
4	Vibrionaceae	1	0	1	1	1
5	<i>Acinetobacter</i> sp.	2	0	0	2	1
5	<i>Alcaligenes</i> or <i>Pseudomonas</i> spp.	2	1	0	2	2
5	<i>Cytophaga</i> spp.	6	3	0	6	6
5	Enterobacteriaceae	3	0	0	3	3
5	<i>Mesophilobacter</i> or <i>Moraxella</i> spp.	2	1	1	2	2
5	<i>Vibrio</i> sp.	1	1		1	1

demonstrates microbial colonization of the surface of the ABCHOW diet after 2 to 4 days immersion. Microorganisms were clearly visible on the surface of the diet, under SEM examination, despite the fragility of the diet. The diet's postimmersion fragility was also expected, especially given dry matter loss of 28% after 48 h immersion (Maguire 1996). This fragility and dry matter loss are noteworthy for two reasons, the first being the relationship between dry matter loss and available surface area. Dry matter loss does not necessarily imply a decline in the surface area. The loss of food particles and the expansion of food particle size following hydration may actually increase the surface area available for microbial colonization. This point should be considered when inter-

preting bacterial density figures. Second, the fragility of the diet postimmersion presented a problem in handling for SEM examination. It may be of benefit to stop the dehydration process at 70% EtOH and store the samples until ready for SEM examination. Samples that were viewed immediately after the dehydration and critical point drying processes were completed, appeared more stable under SEM.

Bacterial numbers observed ranged from 10^2 to 10^6 cells/mm². It is not surprising to find low bacterial numbers on samples from treatments 5 to 7. The diet contains very little moisture (approximately 6%) (Maguire unpublished data) and was stored in a freezer before application to the culture environment. Such conditions will

TABLE 4.

Total fatty acids (g/100 g wt dry wt) for the ABCHOW diet after various periods of immersion, with and without abalone (n = 3).

Treatment	Immersion Time (d)	Abalone Present (+)/Absent (-)	Total Fatty Acids
5	0	-	3.26
1	2	+	3.86
2	2	-	3.98
3	4	+	4.55
4	4	-	4.44

cause many bacteria to become dormant, if not render them unviable. The small increase in bacterial numbers over 4 days immersion in sterile seawater demonstrates the very low initial numbers present.

The bacterial densities observed from treatments 1 to 4 are similar to those reported in the literature. Lewis et al. (1985) reported bacterial numbers in the order 10^4 cells/mm² on crustose coralline algae, a preferred natural settlement substrate of juvenile abalone. The microbial community existing on natural seaweeds is well established and stable, although seasonal fluctuations do occur (Lewis et al. 1985). Bacteria examined in this study have had only 2 to 4 days to colonize the substrate and proliferate. The fact that the microbial biomass is higher on the formulated diet after 4 days than on the abalone's natural diet indicates the suitability of the diet as a substrate for bacteria.

The difference between bacterial numbers observed in treatments 1 and 2 ($p < .05$) (Fig. 2) is a result of the presence of abalone. Bacteria are known to be associated with abalone, both externally and internally (Prieur et al. 1990, Harris 1993). The association of bacteria with abalone places more bacteria in contact with the diet than if the abalone were absent. Abalone may also transfer bacteria between diet pieces. Free-floating bacteria are reliant upon chance contact with the diet before they can locate a suitable substrate (via chemotaxis). Although movement of motile bacteria is relatively fast (Schlegel 1993), their movement is

TABLE 5.

Distribution and total branched-chain fatty acids from the ABCHOW diet after various periods of immersion, with and without abalone.

Branched-Chain Fatty Acids	Immersion Time (d)				
	0	2		4	
		With Abalone	Without Abalone	With Abalone	Without Abalone
i14:0	0.0	0.0	0.0	0.0	0.0
i15:0	0.2	0.2	0.2	0.2	0.2
a15:0	0.1	0.1	0.1	0.1	0.1
i16:0	0.1	0.1	0.1	0.1	0.1
i17:0	0.4	0.4	0.4	0.4	0.4
a17:0	0.0	0.0	0.0	0.0	0.0
i17:1	0.2	0.2	0.1	0.1	0.2
i18:0	0.5	0.5	0.5	0.5	0.5
i18:1	0.1	0.1	0.1	0.1	0.1
br19:1	0.8	0.7	0.7	0.7	0.8
Total	2.4	2.3	2.2	2.2	2.4

Figures represent branched-chain fatty acids as a percentage proportion of total fatty acids (n = 3).

effectively confined to very small areas and does not play any major role in the distribution of bacteria over large areas. However, the water column itself is also an important microbial source, as is indicated by the difference ($p < .05$) in bacterial numbers between treatments 4 and 7. This suggests that bacteria that come into contact with the diet are able to move, via chemotaxis, to a suitable substrate and proliferate.

Bacterial numbers were not significantly different between treatments 3 and 4 ($p > .05$). This apparent loss of treatment effect was brought about by an increase in bacterial numbers in treatment 4 and a decrease in treatment 3 (Fig. 2). It is unlikely that the nutrient content of the diet would be exhausted in such a small sampling time, so it was expected that bacterial numbers would continue to increase over this period. A factor that may have contributed to the decline in bacterial numbers is the presence of protozoan ciliates (6.2×10^2 /mm²) in treatment 3. Ciliates are known to graze heavily on bacteria; indeed, it has been shown that ciliates can clear approximately 10^3 bacteria/ciliate/h (Iriberry et al. 1994, Solic and Krustulovic 1994). This being the case, the ciliates observed on treatment 3 could be capable of consuming 10^5 cells/mm²/h. This figure equates to the whole standing crop of bacteria and thus, may explain how bacterial numbers declined in treatment 3. The abalone themselves may also have been ingesting bacteria from the diet surface or disturbing surface films.

Identification

One of the aims of this study was to categorize, to a degree that allowed an assessment of metabolic activity and capacity, the bacterial microflora colonizing the ABCHOW diet. The majority of the bacteria found on the diet demonstrated an ability to degrade a range of nutrient sources presented to them.

All isolates demonstrated protease activity (Table 3). Although proteins are among the most expensive of the diet's components, their degradation may not be deleterious. Fleming et al. (1996) suggested that protein partially digested by bacteria may be more efficiently digested by abalone. It should be noted, however, that faster protein decomposition generally leads to water quality problems.

The binder was poorly utilized by many of the bacterial isolates (Table 3). This is surprising given that many of the binders used in formulated diets, for example alginate and cellulose, are readily available in the marine environment. If binder is not readily accessible to the bacteria, it may be able to perform its task of holding water-soluble nutrients longer.

Lipase activity was demonstrated by most of the bacterial isolates (Table 3). It is interesting to note, however, that SEM examination of the diet revealed that lipids were not very heavily colonized in relation to other areas (Fig. 6). This indicates that although most of the isolates were able to utilize lipids, they may not have been the preferred nutrient source. The lack of bacterial colonization of lipids seen under SEM examination may be attributed to the hydrophobic/hydrophilic interactions that must be overcome by the bacteria at the lipid/water interface.

Because many of the oxidative organisms were unable to produce acid from glucose, it is reasonable to suggest that they are likely to be poor users of complex carbohydrates, which are initially hydrolyzed to glucose. No isolates identified as *Pseudomonas* or *Acinetobacter* were able to use the carbohydrate source supplied. Abalone consume a natural diet that is high (40–50%) in carbohydrates and possess many enzymes capable of carbohydrate hydrolysis (Fleming et al. 1996). As a result, many formulated

TABLE 6.

Straight-chain fatty acids from the ABCHOW diet after various immersion times with and without abalone.

Straight-Chain Fatty Acids	Immersion Time (d)				
	0	2 With Abalone	2 Without Abalone	4 With Abalone	4 Without Abalone
Saturated					
12:0	0.1	0.1	0.1	0.1	0.1
14:0	4.1	4.2	4.1	4.2	4.2
15:0	0.4	0.4	0.4	0.4	0.4
16:0	17.4	17.9	18.0	18.1	18.2
17:0	0.2	0.2	0.2	0.2	0.3
18:0	3.2	3.2	3.3	3.3	3.3
Total	25.4	26.0	26.1	26.3	26.5
Monoenoic					
16:1(n-9)	0.2	0.1	0.1	0.2	0.2
16:1(n-7)	4.4	4.5	4.5	4.6	4.8
18:1(n-9)	10.6	10.6	10.9	11.0	11.0
18:1(n-7)	1.8	1.8	2.0	2.0	2.1
18:1(n-5)	0.2	0.2	0.2	0.2	0.2
Total	16.2	17.2	17.7	17.8	18.3

Figures represent straight-chain fatty acids as a percentage proportion of total fatty acids ($n = 3$).

abalone diets comprise up to 60% carbohydrate. The bacteria associated with surface of abalone would also be expected to perform well on carbohydrates, but this does not seem to be the case. It is unlikely, then, that the predominantly oxidative isolates in this study would have a great effect on carbohydrate availability during 4 days immersion.

Fatty Acid Analysis

The quantitative increase in total fatty acids is thought to have resulted from a decrease through leaching in amounts of other food components. Many lipids are not water soluble, so a rise in lipid content and total fatty acids (g/100 g dry wt), as water-soluble nutrients are lost, is expected.

Many Gram-negative bacteria, including: *Pseudomonas*, *Alteromonas*, *Moraxella*, *Cytophaga*, and *Flavobacterium* (Kaneda 1991), all of which have been isolated from the ABCHOW diet, contain greater than 20% branched-chain fatty acids. It has been noted, however, that branched-chain fatty acids are more common in Gram-positive bacteria; whereas, straight-chain fatty acids are more common in Gram-negative bacteria (Kaneda 1991). Bacterial growth on the diet should, then, be indicated by an increase in

these fatty acids. No such change was demonstrated in the present study.

SEM analysis has demonstrated clearly that bacterial colonization of the diet occurs after immersion. Chemotaxonomic techniques have previously been utilized for ecological studies investigating relatively low-nutrient environments, so very small shifts in the fatty acid spectrum have been observable. However, the chemotaxonomic method chosen failed to demonstrate an obvious increase in microbial biomass. The most likely explanation for this is that it is inadequate for use on samples of high original lipid content. Table 5 indicates that the ABCHOW diet was relatively high in initial fatty acid levels. This high lipid content masks the presence of bacterial lipids, which make up only a very small percentage of total lipids.

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ABUNDANCE, RECRUITMENT, AND MORTALITY OF PACIFIC LITTLENECK CLAMS *PROTOTHACA STAMINEA* AT CHUGACHIK ISLAND, ALASKA

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ABSTRACT From 1992 to 1996, the Alaska Department of Fish and Game annually surveyed the Pacific littleneck clam *Protothaca staminea* (Conrad, 1857) population at Chugachik Island, Alaska. Estimates based on randomly placed survey quadrats indicated the population declined from 7.2 million clams in 1992 to 3.3 million clams in 1995 and increased to 5.5 million clams in 1996. Survey biomass estimates similarly declined from 136,887 kg in 1992 to 65,852 kg in 1995, and then increased to 115,495 kg in 1996. Annual harvest biomass, mean weight-at-age, and survey abundance and age composition data from 1992 to 1996 were also used as inputs in an age-structured model of the Chugachik clam population. The age model used independent selectivity curves to estimate age-specific recruitment to the fishery and the survey. Age of 50% selectivity to the fishery ranged from 7 to 11 years, depending upon model emphasis of survey age composition data. This agreed well with size-at-age data, indicating few clams recruited to legal harvest size of 38 mm prior to age 5 and only 50% had recruited by age 7. Length-based estimates of annual recruitment to legal size ranged from 6 to 12% of the population, averaging 10%. Greater age model emphasis on survey age data generally increased both estimated survival and the age when 50% of a cohort recruited into the fishery, and decreased the age when 50% of a cohort recruited into the survey. Age-model estimates of population abundance also varied with weighting applied to survey age composition data. Population abundance trends from the model agreed well with survey trends from 1992 to 1995, although the model usually exceeded survey estimates. Model estimates were 15 to 25% less than survey estimates for the 1996 population, probably because of a lag in model response to abundance trend changes.

KEY WORDS: Littleneck clams, *Protothaca staminea*, age-model, Alaska

INTRODUCTION

Hardshell clams have long been an important component of the recreational and commercial fisheries in Cook Inlet, Alaska. The commercial fishery dates to the 1950s, when butter clams *Saxidomus giganteus* (Deshayes, 1839) were sold in canned and fresh markets. Sales of canned clams contaminated with paralytic shellfish poisoning (PSP) from southeast Alaska subsequently led to a market collapse in the late 1950s. The commercial hardshell clam fishery in Cook Inlet re-emerged, targeting Pacific littleneck clams *Protothaca staminea* (Conrad, 1857) in 1986 after the Alaska Department of Environmental Conservation (DEC) certified the Chugachik Island beach in Kachemak Bay, Alaska, for commercial harvesting. Commercial harvests during 1986 to 1991 averaged 7,107 kg of clams annually from Chugachik Island (Fig. 1). In addition, recreational diggers accounted for 77% of all hardshell clam harvests in southern Cook Inlet from 1986 to 1996, although beach-specific harvest information is unavailable (Scott Meyer, Alaska Department of Fish and Game, Homer, Alaska, unpublished data).

The Alaska Department of Fish and Game (ADF&G) initiated annual surveys on Chugachik Island in 1992, because this area had the longest history of DEC certification for commercial clamming in Kachemak Bay. Average harvests increased to 14,876 kg annually during 1992 to 1994 (Gustafson 1995). The commercial fishery was closed in 1995 and 1996 after ADF&G surveys indicated 3 consecutive years of abundance declines. Management strategies for the commercial hardshell clam fishery in Kachemak Bay now include: minimum legal size of 38 mm (1.5 inches); 1 April annual registration deadline; fishing district segregation into two groups that open on alternate years; quarterly harvest allocations with maximum quotas for specific beaches; closures in areas of high recreational use; and closures during 1 November to 15 March

when ambient air temperatures are below freezing and during weekends from 15 May to 15 September.

To evaluate commercial fishery impacts on Pacific littleneck clams at Chugachik Island, we used survey data in a length-based model to estimate age-specific recruitment to legal size. Our field surveys were designed to estimate legal clam abundance in the study area. Most clam studies wash the removed substrate through screens (Paul and Feder 1973). However, because our budget and survey time was limited, we did not wash the removed substrate through screens. As a result, as the survey design developed, we were able to sample more quadrats during a site visit, but with the recognition that our survey was biased toward larger clams because of a lack of substrate screening. To back-calculate the sub-legal component of the population and estimate the true clam population abundance, recruitment, and mortality, we developed an age-structured model that accommodated survey bias through selectivity functions.

MATERIALS AND METHODS

Survey Data

Abundance, biomass, and age composition of the hardshell clam population at Chugachik Island in Kachemak Bay, Alaska were estimated from surveys conducted in May during 1992 to 1996 (Gustafson 1995). The littleneck clam bed, defined to include habitat located between the -1.5 m mean low water level and the blue mussel *Mytilus edulis* (Linnaeus, 1758) bed, was estimated to encompass 61,254 m². Substrate in the clam bed was a mixture of 1 to 8-cm coarse rock and muddy sand (Gustafson 1996). During tides exposing the beach to the -1.5 m mean low water level, substrate was removed by hand digging with a rake to a depth of 30 cm within randomly placed quadrats measuring 0.5 m × 0.5 m. The number of quadrats sampled ranged annually from 12 in 1992

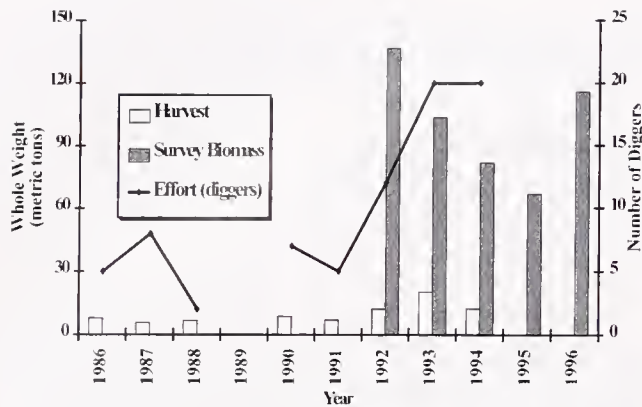


Figure 1. Estimates of survey biomass and commercial fishery harvest and effort for Pacific littleneck clams at Chugachik Island, Alaska, 1986 to 1996.

to 35 in 1995 (Table 1). During substrate removal and replacement, all observed clams were retained. Clams were transported to the laboratory to obtain age, weight, and length data; weight-at-age was estimated from samples collected in 1997 only. Clams were aged by counting concentric growth rings on the external surface of the clamshell. The use of these rings to indicate annual growth in littleneck clams has been validated through mark-recapture and size distribution studies (Houghton 1973, Paul and Feder 1973). Although cautioning against the use of growth lines as annuli for littleneck clams in a clean-sand habitat, Peterson and Ambrose (1985) found that specimens deposited a single growth line over 12 months in a muddy-sand environment, as is found at Chugachik Island. All age and size-at-age data used in the model were derived from survey samples.

A simple random sample design was used to estimate littleneck clam abundance at Chugachik Island. Abundance was calculated by multiplying the mean density of littleneck clams in sample quadrats (0.25 m²) by the total area where littleneck clams were found. Standard variance estimates for simple random sampling were used to calculate the variance (Cochran 1977). The sampling fraction of quadrats was less than 1% at Chugachik Island. Finite population corrections were not included in the variance estimate of abundance, because finite population corrections can generally be ignored if the sampling fraction does not exceed 5% (Cochran 1977).

We estimated annual fishery recruitment using length-at-age survey data and assuming knife-edged recruitment at the minimum legal size for the fishery. For the length-based model we calculated

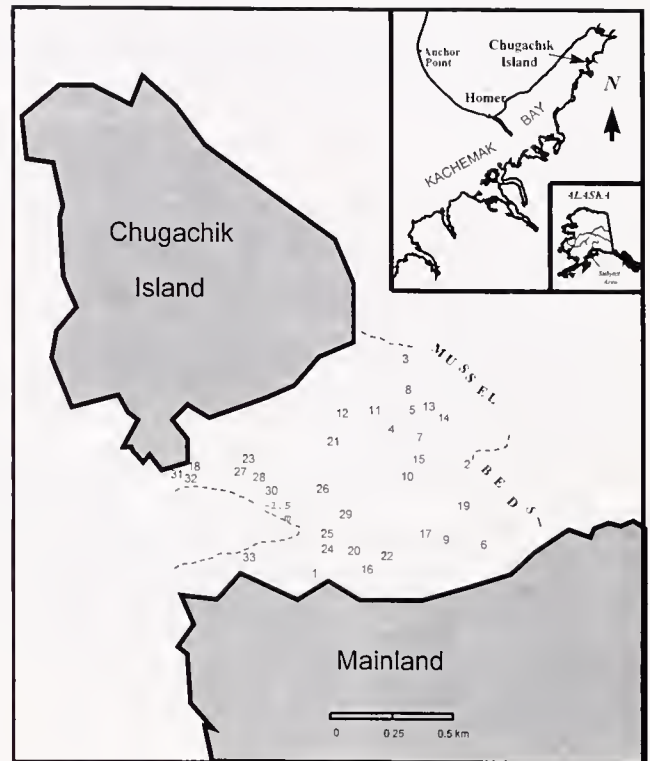


Figure 2. Study area showing sequential quadrat selection during the 1996 survey as an example of the simple random survey design at Chugachik Island, Alaska.

recruitment as differences in mean percentage legal clams by age class. For age class a , the mean proportion of legal clams p_a among survey years was calculated from the following:

$$p_a = \frac{\sum_{y=1}^k l_{a,y}}{\sum_{y=1}^k n_{a,y}}, \quad (1)$$

where $l_{a,y}$ is the number of legal clams age a clams in year y , and $n_{a,y}$ is the abundance of age a clams in year y . Mean annual rate of recruitment to age class a was estimated as the difference between the proportions of legal clams in ages a and $a-1$ using the following:

$$r_a = p_a - p_{a-1}. \quad (2)$$

TABLE 1.

Estimates of annual survey abundance and length-based recruitment for Pacific littleneck clams at Chugachik Island, 1992 to 1996.

Year	Sample Quadrats n	Sample Densities		Annual Abundance		Annual Recruitment	
		Mean (Clams/m ²)	SD	(Clams)	95% C.I.	(Clams)	Percentage
1992	12	117.7	62.98	7,207,502	±2,923,930	858,548	11.9%
1993	16	89.8	52.68	5,497,507	±1,767,208	463,089	8.4%
1994	33	79.3	74.35	4,888,737	±1,791,592	278,764	5.7%
1995	35	53.3	38.24	3,262,213	±1,021,592	369,098	11.3%
1996	33	88.2	67.62	5,405,201	±1,886,510	628,649	11.6%
Mean	26	85.7		5,252,232		519,630	9.9%

The total year y recruitment R_y was the cumulative products of age class abundance and age-specific recruitment rates as in the following:

$$R_y = \sum_{a=1}^m (r_a \times n_{a,y}). \quad (3)$$

Age-Structured Model

Our primary objectives in using an age-structured model were to estimate natural mortality, fishery selectivity, survey selectivity, and annual population abundance for 1992 to 1996. Information supplied to the age model included survey data and commercial harvest data. Commercial harvest weights were obtained from ADF&G fish tickets during 1992 to 1994 when the littleneck clam fishery occurred. Age-structured models that incorporate heterogeneous data have been reviewed by Hilborn and Walters (1992), Megrey (1989), and Quinn and Szarzi (1993). The Chugachik model incorporated auxiliary information, similar to age-based models developed by Deriso et al. (1985). In our conceptual model of the annual cycle of events affecting Pacific littleneck clams at Chugachik Island (Fig. 3), age increments occur at the end of winter to coincide with the approximate time of annulus formation. The population is then subjected to age-specific mortality through commercial fishing, followed by natural mortality prior to again incrementing to the next year class.

The Chugachik Island resource also incurs unquantified, recreational and subsistence harvests. It is likely that strong year classes, once recruited to legal size, are subjected to greater non-commercial mortality than weak year classes. Although noncommercial mortality abundance varies annually, annual mortality rates may be moderately stable, because the abundance removals from strong year classes are greater than abundance removals from weak year classes. Thus, the proportion of clams dying from non-commercial sources is assumed to be stable among years and is treated as natural mortality. Natural mortality is described by a single exponential decay function for all years and cohorts. Our age-structured model used a reduction equation to describe annual

survival. The number of age- a clams in a cohort in the spring of year y after winter annulus formation was the following:

$$\hat{N}_{a+1,y+1} = S(N_{a,y} - C_{a,y}) \quad (4)$$

where S is the annual survival rate, a model-estimated parameter, and $C_{a,y}$ is commercial fishery harvest. The population model assumes that clams from age 2 to 14 are present in the estimated population. Although age classes outside this range were observed in all Chugachik surveys, clams younger than age 2 have not consistently appeared in surveys, and cohorts older than age 14 are a minor component of the population. Age 2 recruitment in 1996 was calculated as the median of age 2 clam abundance estimates for the 1992 to 1995 population years.

Through independent logistic functions describing fishery and survey selectivity, the model accommodated differences among age compositions of the underlying population, the field surveys, and the commercial fisheries. Relationships between clam age and fishery and survey selectivity were assumed to be constant among years. Annual age compositions in the commercial fishery were estimated by the age-structured model, because commercial harvests were not sampled. Composition of the annual commercial harvest $f_{a,y}$ was estimated from an age-specific selectivity function s_a and model-estimated cohort abundance using the following:

$$f_{a,y} = \frac{s_a \hat{N}_{a,y}}{\sum_{a=2}^{14} [s_a \hat{N}_{a,y}]}, \text{ and} \\ s_a = \frac{1}{1 + e^{\beta(a-\alpha)}} \quad (5)$$

where α was the age of 50% selectivity, and β was a steepness parameter. Given the fishery selectivity function and mean weight-at-age, we calculated the number of clams that produced the observed harvest biomass for each calendar year.

Survey selectivity was similarly described by the following logistic function.

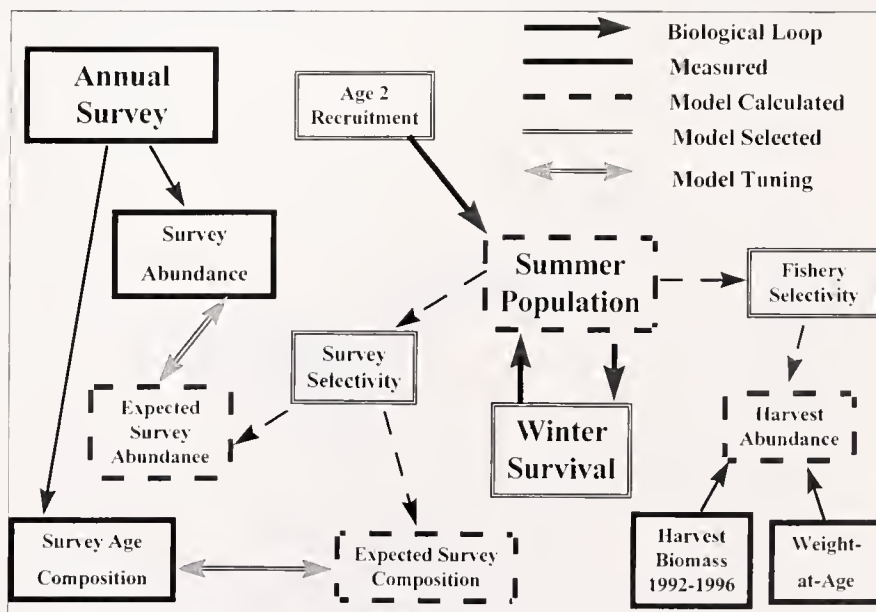


Figure 3. Components of the age-structured model used to evaluate the Pacific littleneck clam population at Chugachik Island, Alaska.

$$p_a = \frac{1}{1 + e^{\phi(a-\tau)}} \quad (6)$$

where τ was the age of 50% selectivity by the survey gear, and ϕ was a steepness parameter.

Measurement errors in each of the data sources are assumed to be independent. We also assume the model is correctly specified with respect to the amount and type of available data so that parameter estimates are not correlated and differences between model estimates and observed values are caused by measurement error, not errors in correctly specifying mathematical forms of the underlying processes. This age-structured model was applied to a variety of survey and fishery size and abundance data measured in different units and of varying utility in identifying true parameter values. Nonlinear least-squares techniques were used to minimize sums of squares constructed with heterogeneous auxiliary data from the Chugachik Island population. Unlike least-squares linear regression, there is no rigid statistical theory underlying the parameter estimation procedure. The rationale is that the best estimates of model parameters should provide a reasonable fit to all available data. In some cases, data are arc sine-transformed to achieve symmetric and approximately normal error distributions, although robustness of parameter estimates to departures from normality is unknown (Funk 1994). However, various weighting scenarios were applied to error terms from data sources to examine the utility of these data in the model.

One measure of age-structured model fit was obtained by comparing annual age compositions observed by the surveys to those estimated by the model. The sum of squares SSQ_{survey_age} measured the goodness of fit of the age composition of the survey and was computed as follows:

$$SSQ_{survey_age} = \sum_y \sum_a (\sin^{-1} \sqrt{p_{a,y}} - \sin^{-1} \sqrt{\hat{p}_{a,y}})^2 \quad (7)$$

where $\hat{p}_{a,y}$ was the model estimate and $p_{a,y}$ the survey observation of the proportion in year y comprised by age a . To stabilize the variance, the age compositions were transformed by taking the arc sine of the square root of the proportions. The fishery age composition was fit across ages 2 to 14 and years 1992 through 1996.

The age model also minimized the sums of squares between model-estimated abundances and survey-estimated population abundances. The sum of squares was calculated by the following:

$$SSQ_{abund} = \sum_{y=1992}^{1996} [\ln(\hat{N}_{y,survey}) - \ln(\hat{N}_{y,model})]^2$$

where $N_{y,survey}$ was the survey estimate and $N_{y,model}$ the model estimate of abundance in year y . We used the natural log of clams numbers because a log-normal error structure is commonly associated with abundance data (Funk 1994).

Model sensitivity was examined through several scenarios that varied the emphasis, or weighting, on available data sources. In some cases, model runs were rejected, because they yielded unrealistic results, such as the age of 50% selectivity being greater than 15 or a population abundance estimate that was negative.

RESULTS AND DISCUSSION

Survey and Length-Based Estimates

Survey estimates showed population abundance declined from 7.2 million clams in 1992 to 3.3 million clams in 1995 before staging a moderate increase to 5.4 million clams in 1996 (Table 1, Fig. 4). Survey biomass similarly declined from 136,887 kg in 1992 to 65,852 kg in 1995, and then increased to 115,495 kg in 1996.

Based on size-at-age of littleneck clams among all survey years, the length-based model indicated that few clams recruited to legal size prior to age 5 (Fig. 5). Age-specific clam recruitments were 0.4% for age 5, 5.8% for age 6, 44.3% for age 7, 39.1% for age 8, 9.2% for age 9, and 1.1% for age 10. Cumulative increases in the legal component of the surveyed population indicated age 7 to be the age of 50% recruitment to the commercial fishery. Application of age-specific mean recruitment to estimated annual age composition produced annual recruitment rates ranging from 5.7% to 11.9% and averaging 9.9% during 1992 to 1996 survey time series (Table 1). Annual recruitment as a percentage of the total abundance declined during 1992 to 1994, the years of the com-

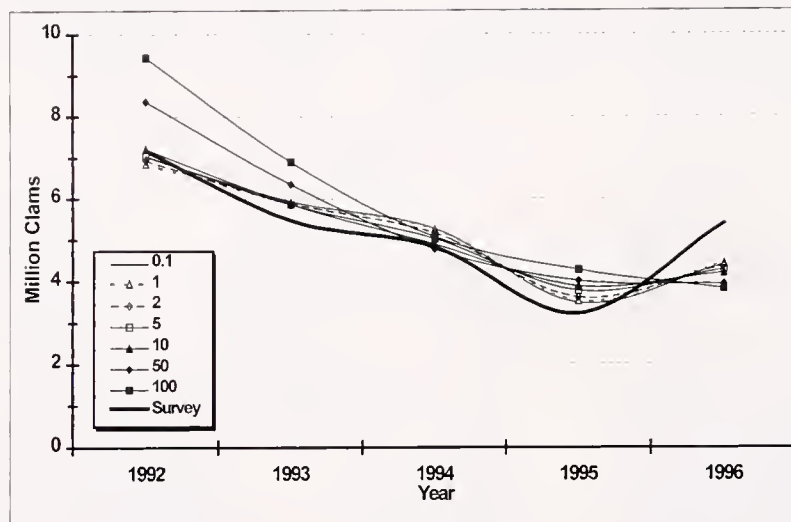


Figure 4. Comparison of Pacific littleneck clam abundance estimates from field surveys (thick line) and from an age-structured model with different weighting of the survey age composition data, Chugachik Island, Alaska, 1986 to 1996.

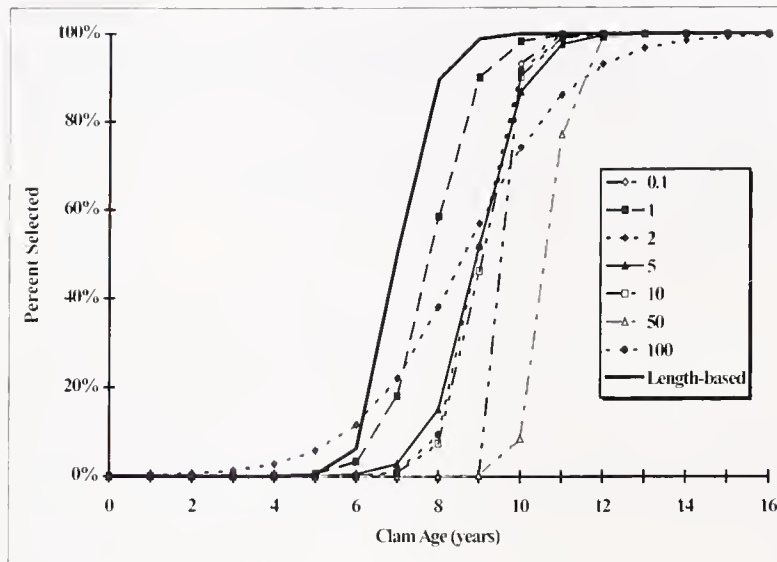


Figure 5. Fishery selectivity for Pacific littleneck clams calculated from a length-based model (thick line) and from an age-structured model applying different weights to survey age composition data.

mercial fishery, and increased in 1995 and 1996 when the fishery was closed.

Age-Structured Model Estimates

An age-structured model was previously used to estimate sustained recreational fishery yield for Pacific razor clams *Siliqua patula* (Dixon, 1788) in Cook Inlet (Quinn and Szarzi 1993). This model relied heavily on fecundity data and spawner-recruit relationships. In contrast, our approach for Chugachik clams was more generic in dealing with known commercial harvests but unknown recreational removals to evaluate the underlying population abundance. Although model estimates of the Chugachik Island clam

population varied with weighting applied to survey age composition data, results agreed well with the 1992 to 1995 population decrease observed in surveys (Fig. 4). For most weighting options, model estimates of the population slightly exceeded survey estimates. This supports the assumption of systematic survey selectivity. Some studies both within and outside of the Cook Inlet area have attempted to reduce selectivity by passing the removed substrate through mesh screens to reduce the clam nondetection (Gustafson 1996). However, using screens also decreases the number of sample quadrats that can be dug. Through the logistic function, our model accommodates systematic survey selectivity that results from a greater sample rate but with slightly less scrutiny of quadrats. The primary exception to model estimates slightly exceeding survey estimates was 1996, when all model runs suggested

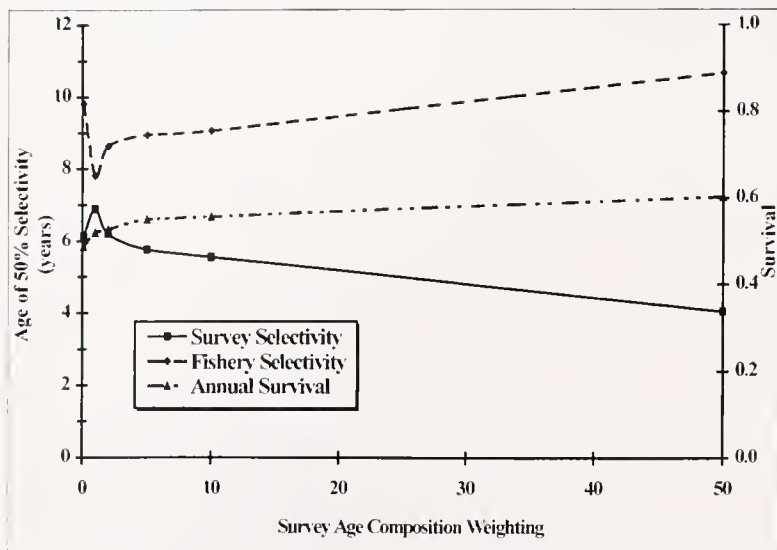


Figure 6. The effect of increased weighting of survey age composition data on the estimated annual survival and the ages of 50% selectivity in the commercial fishery and in abundance surveys for Pacific littleneck clams at Chugachik Island, Alaska.

the true population was 15 to 25%, or up to 1.0 million clams, less than the survey estimate. Because the model tracks abundance throughout the life of a cohort from the age of recruitment to the survey or fishery, a long time series is typically required to follow changes in population trends reliably. Thus, the model may lag behind trend changes detected by the survey. The lack of model response to the 1996 increase could reflect either a response lag or errors in survey estimation. Interestingly, preliminary results from the 1997 survey showed a slight increase over the 1996 survey estimate.

The age model estimated that about 50% of a cohort recruited into the fishery at ages ranging from 7 to 11 years, depending upon weighting applied to survey age composition data (Fig. 5). In general, increased weighting resulted in increases to estimated survival and age of 50% selectivity by the fishery, and decreased the age of 50% selectivity by the survey (Fig. 6). Annual survival generally ranged from 50 to 60%. The age of 50% selectivity in the fishery exceeded that in the survey by 1 to 6 years, a result consistent with field observations.

In summary, the age-structured model estimates agreed moderately well with length-based model estimates. The age model

was found to be sensitive to starting parameters and some anomalies observed in model run results were probably attributable to inappropriate initial parameters. Fit of an age-structured model to available data may improve if: (1) age composition estimates of the commercial harvests were based on commercial fishery samples rather than model estimates; and (2) noncommercial harvests could be separated from natural mortality.

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REPRODUCTIVE CYCLE OF THE GIANT REEF CLAM *PERIGLYPTA MULTICOSTATA* (SOWERBY, 1835) (PELECYPODA: VENERIDAE) AT ISLA ESPIRITU SANTO, BAJA CALIFORNIA SUR, MEXICO

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ABSTRACT The reproductive cycle of *Periglypta multicostata* (S.), was studied at Isla Espíritu Santo, Gulf of California, México, from October 1992 to December 1993. The reproductive activity was present throughout the study period, except in February 1993, nevertheless a distinct seasonality was observed with three distinct peaks of spawning activity. A clear relationship between spawning and temperature or photosynthetic pigments concentration was not observed. Spawning occurs all year, but at a lower rate in the months with the lowest water temperatures.

KEY WORDS: Reproductive cycle, gametogenesis, bivalves, Veneridae, *Periglypta*

INTRODUCTION

The giant reef clam, *Periglypta multicostata* (Sowerby, 1835), is the heaviest if not the largest of the Panamic members of the family Veneridae. They inhabit the sand among rocks at extreme low tide from Gulf of California to Peru (Keen 1971). This species is a dominant component of the zone of the coral *Pocillopora elegans* in the rocky substrata communities of Isla Espíritu Santo and coexists with three bivalve species, *Ventricolaria isocardia*, *Megapitaria aurantiaca*, and *Chione tumens*.

In Baja California Sur, wild stocks of giant reef clams remain practically untouched and are considered as a potential fisheries resource; however, aquaculture is not recommended (Baquero 1989). No research has been done on the biology or life history of this species. Clams are gathered by free diving and are dug out with hands, knives, or forks.

The synchronization of reproductive activity in local populations is very important for successful fertilization. Reproduction seems to be cyclic, with events coordinated on an annual cycle (Eversole 1989). Environmental factors may influence the timing of reproduction in clams. The most commonly cited are food availability and temperature (Bayne and Newell 1983, MacDonald and Thompson 1985, Jaramillo et al. 1993). The water temperature and its variation with latitude is used by many authors to attempt to explain reproductive timing in bivalves (Newell et al. 1982, Lozada and Bustos 1984, Manzi et al. 1985, Malachowski 1988, Hesselman et al. 1989, García-Domínguez et al. 1993). The food availability is used to attempt to explain spawning timing in bivalves in the sense of that if the spawning coincides with the highest food availability, this enables the larvae to exploit the phytoplankton bloom (Jaramillo et al. 1993, Villalejo-Fuerte et al. 1996a).

The lack of biological information for proper management has led to overexploitation and misuse of stocks. It is essential to know the life cycle of the target species, and documentation of the reproductive cycle in a fishery is one necessary step in determining when recruitment might occur. This study describes the reproductive cycle and the spawning season of *P. multicostata* in relation to the temperature and food availability.

MATERIAL AND METHODS

Monthly, 20 to 25 specimens of a wild and unexploited population of *P. multicostata* were collected from October 1992 to December 1993 at Isla Espíritu Santo, Bahía de La Paz, Gulf of California, México (110°24'27"W, 24°28'54"N) by a scuba diver at 3- to 6-m depth. A total of 310 organisms were captured. When the biological samples were collected, water temperature was recorded. The photosynthetic pigment concentration (mg chlorophyll/m³) in Bahía de La Paz, Gulf of California was obtained from satellite-derived information (Trant et al. 1993), this was considered to be an estimation of the food availability for the clams.

Mantle, adductor muscles, gills, labial palps, and siphons were removed, keeping only the visceral mass (gonad, liver, and gastrointestinal tract) and the foot. These tissues were fixed in buffered 10% formalin. A slice of tissue of each clam was obtained from the dorsal area of the visceral mass and embedded in paraffin. Sections 7- to 9- μ m thick were stained with hematoxylin and eosin (Luna 1968). This method was adopted after verifying, in 25 specimens, that gonadal maturity was uniform in different parts of gonad.

The reproductive process (either spermatogenesis or oogenesis) of *P. multicostata* was categorized in five stages based solely on morphological observations, characterized by the structure of the gonad, presence, absence, and quantity and development of gametes (Table 1, Figs. 1, 2, 3). In all the stages of gonadal development, phagocytes were present in varying proportions.

Individuals were sexed by microscopic examination of histological slides. Sex ratios were analyzed with chi-square to test the significance of the deviation from the expected sex ratio of 1:1, for the total sample. The indifferent stage clams were not considered.

Mean oocyte diameters, and their standard deviation, of six females selected randomly per month were determined from histological sections using an eyepiece graticule calibrated with a stage micrometer. At least 100 oocytes sectioned through the nucleus (i.e., near the maximum diameter) per individual were measured along the longest axis. Individuals with few measurable oocytes and extensive phagocytosis ("spent" specimens) were not

TABLE 1.
Developmental Stages of *P. multcostata* Gonads.

Maturity Stage	Female	Male
Indifferent	Characterized by presence of acinis with total absence of gametes. It is not possible to distinguish the sex. The connective tissue is abundant.	
Developing	Oocytes inside of follicles are conspicuous, young oocytes with pear shape growing attached to follicular walls. The area of connective tissue decreasing.	A variable quantity of germinal cells and spermatozoa were present inside the follicles. The area of connective tissue decreasing.
Ripe	Free large oocytes present in the lumen with maximum size, few oocytes with pear shape attached to follicular walls. Connective tissue absent.	Follicles filled with spermatozoa. Other spermatogenic cells restricted to a thick layer on the follicular walls. Connective tissue absent.
Partially spawned	Follicles containing some oocytes and large spaces, while others were empty. Some connective tissue visible.	Follicles partially empty. A marked decrease in the number of spermatozoa filling the lumen. Some connective tissue visible.
Spent	Few residual oocytes, being phagocytized by amoebocytes. No evidence of active oogenesis.	Follicles collapsed, amoebocytes phagocytizing residual spermatozoa. No evidence of active spermatogenesis.

considered, using the criteria of Grant and Tyler (1983a) and Grant and Tyler (1983b).

RESULTS

Spawning activity was present throughout the study period, except in February 1993 (Fig. 4). Nevertheless, there seems to be a distinct seasonality in the reproductive cycle, because *P. multcostata* has fluctuations in its reproductive intensity, showing three distinct peaks of spawning activity during the study period: October to December 1992, July to September 1993, and November to December 1993.

Giant reef clams in the indifferent stage were observed every month except October 1992. Developing clams were present over the year except in February, September, November, and December 1993. The highest frequency of ripe organisms was present in October 1992, March, June to September, and November 1993. Partially spawned individuals were observed all year, except in

February. The highest frequency of partially spawned individuals was observed from June to September. The spent stage was recorded throughout the year, except in April.

Of 310 clams examined, 149 (48.1%) were females and 83 (26.8%) males. The remaining 78 (25.1%) were undifferentiated. The sex ratio of the sexed sample (1.8 F: 1 m, $n = 232$) differs significantly ($p \leq .05$) from the expected ratio of 1:1.

During the year, the mean oocyte diameter was $> 47 \mu\text{m}$, except in October, when it was $41 \mu\text{m}$ (Fig. 5). In February, all the clams were spent and indifferent, so there were no oocytes. The pattern observed in oocyte diameters was consistent with the histological observations, which suggests spawning throughout the year, with the exception of February 1993. The standard deviations were wide in all months, indicating the presence of both small and large oocytes characteristics of ripe and spawning stages.

The water temperature during the study period varied from 22°C to 31°C . The highest values were in October 1992 and August 1993, and the lowest values were in February and March 1993 (Fig. 5).

Photosynthetic pigment concentration ($\text{mg chlorophyll/m}^3$) in Bahía de La Paz was greater in the colder months than in the warmer ones. The maximum value was in January ($4.87 \text{ mg chlorophyll/m}^3$), and the minimum was in September ($1.36 \text{ mg chlorophyll/m}^3$) (Fig. 5).

DISCUSSION

The annual reproductive cycle of *P. multcostata* at Isla Espíritu Santo showed seasonality, with a protracted period of reproduction indicated by the consistent presence of spawning activity throughout the study period, with the exception of February 1993. Other species of bivalves, abundant in this locality, such as *Megapitaria aurantiaca* (García-Domínguez et al. 1994) or *Pinctada mazatlanica* (García-Domínguez et al. 1996), have no seasonal reproductive cycles, and their spawning activity is continuous. In other bivalves, such as *Mercenaria* spp., the spawning is essentially continuous in lower latitudes, but there are still cycles (Hesselman et al. 1989).

Among other venerid clams from other localities along the Mexican Pacific coast, several other species lack distinct seasonal

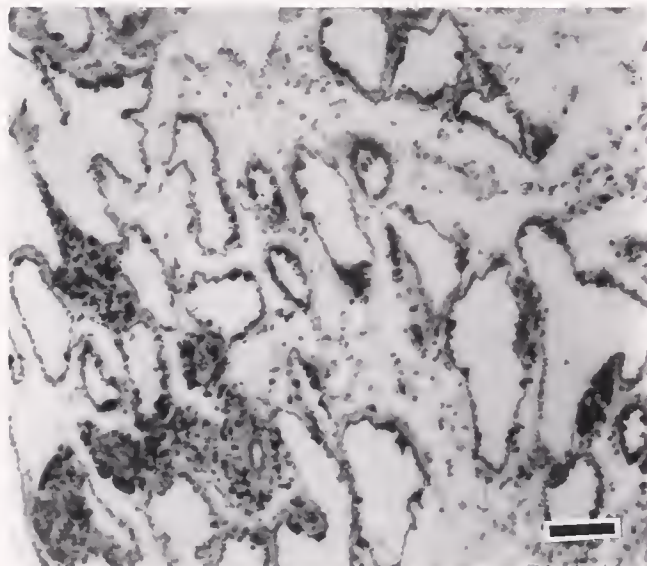


Figure 1. Indifferent stage; scale bar = $50 \mu\text{m}$.

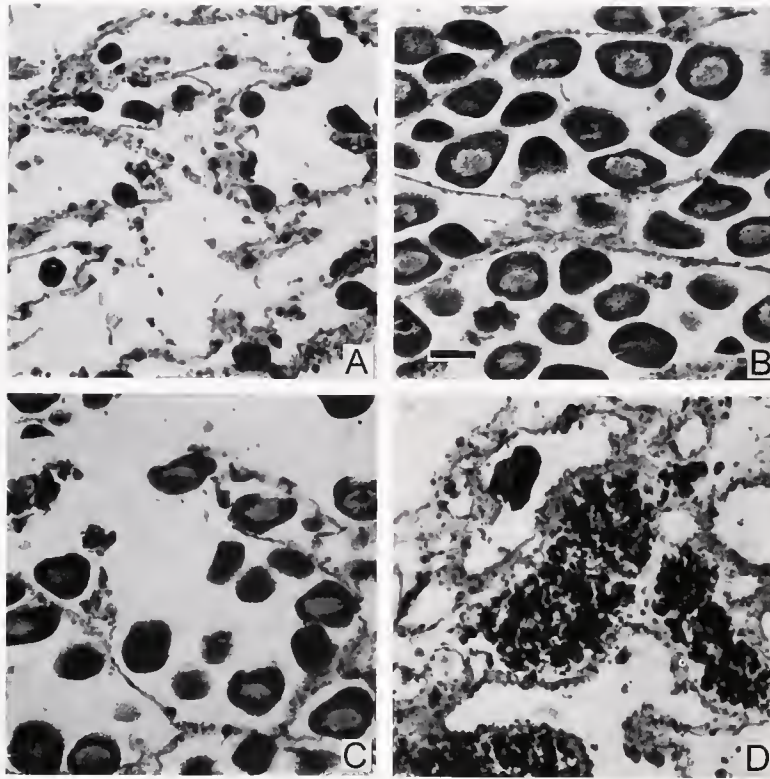


Figure 2. Photomicrographs of gonadal stages of the female giant reef clam, *P. multicostata*. (A) developing stage, (B) ripe stage, (C) partially spawned stage, and (D) spent stage; scale bar = 50 μ m.

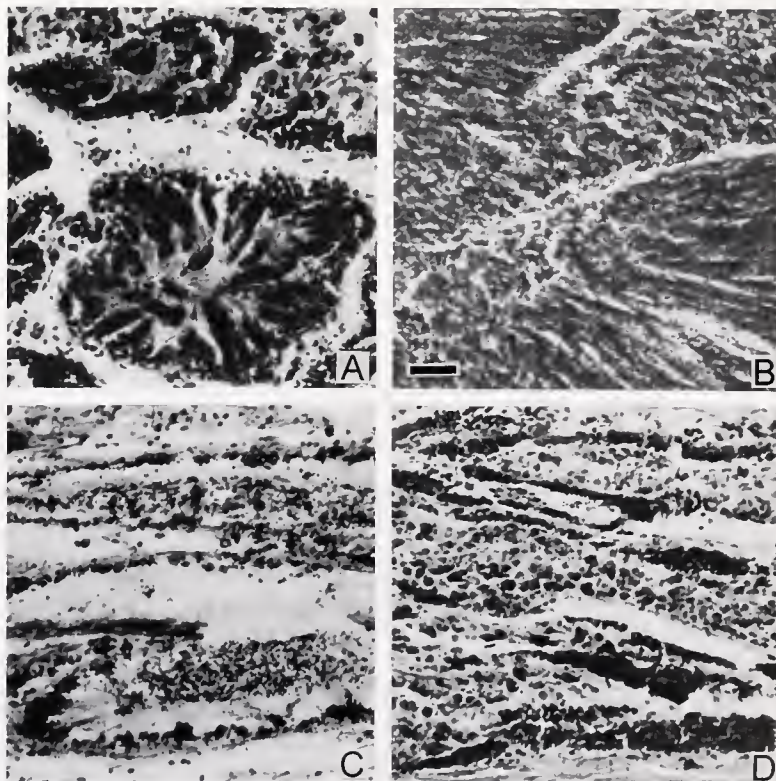


Figure 3. Photomicrographs of gonadal stages of the male giant reef clam, *P. multicostata*. (A) developing stage, (B) ripe stage, (C) partially spawned stage, and (D) spent stage; scale bar = 50 μ m.

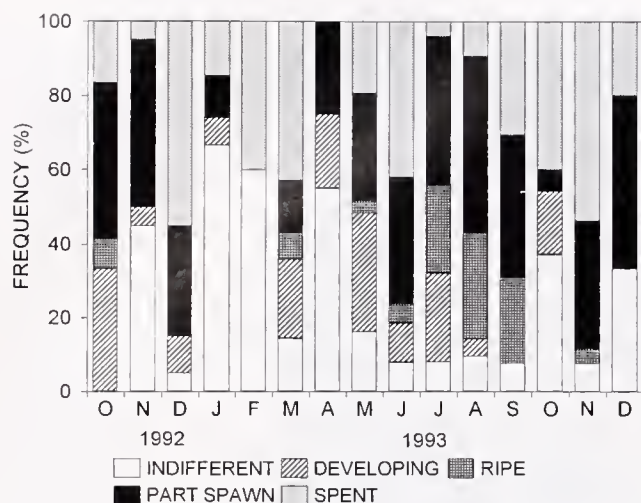


Figure 4. Reproductive cycle of *P. multicostata* at Isla Espíritu Santo. Relative frequency of gonadal stages between October 1992 and December 1993. Observations of males and females are combined.

reproductive cycles; *Megapitaria aurantiaca*, *M. squalida*, *Dosinia ponderosa* (Baquero and Stuardo 1977), *Chione undatella* (Baquero and Masso 1988), and *M. squalida* (Villalejo-Fuerte et al. 1996b). Other bivalves, such as *Mercenaria mercenaria*, displayed a synchronized polymodal breeding pattern, although not every year. In some years, it is polymodal and in others, it is bimodal with continuous spawning (Heffernan et al. 1989).

The sex ratio of the sample differs significantly from the expected ratio of 1:1, with females being dominant, which suggests females outnumbered males in the population. The same was observed for the pearl oyster *P. mazatlanica* in the same locality (García-Domínguez et al. 1996), this condition may be related to the fact that *P. mazatlanica* is a protandric hermaphrodite (Sevilla 1969, Saucedo and Monteforte 1994, García-Domínguez et al. 1996). However, in the case of *P. multicostata*, evidence of hermaphroditism was not observed. Also, the fact that within the population as a whole, the majority are females is considered typical of freshwater and brackish water bivalves (Morton 1985).

Oocyte diameters reflect the gametogenic cycle, thus minimum diameters coincide with the developing stage, and maximum diameters coincide with the mature and partially spawned stages. This pattern is similar for other species such as *Argopecten circularis*, *Glycymeris gigantea*, and *Laevicardium elatum* (Villalejo-Fuerte and Ochoa-Báez 1993, Villalejo-Fuerte et al. 1995, Villalejo-Fuerte et al. 1996a).

MacDonald and Thompson (1985) suggested that bivalve gamete production is strongly influenced by such environmental factors as temperature and food availability set in a seasonal context. The reproductive cycle of *Periglypta multicostata* was not clearly related to the water temperature. The same has been observed for such other venerid clams as *Megapitaria aurantiaca*, *M. squalida*, and *D. ponderosa* from Bahía Zihuatanejo (Baquero and Stuardo 1977), *M. aurantiaca* from Isla Espíritu Santo (García-Domínguez et al. 1994), and in other bivalves such as *Pinctada mazatlanica* (García-Domínguez et al. 1996) from Isla Espíritu Santo. The relation between the temperature and spawning of other bivalves of the Mexican Pacific coast has been well documented in several species; *Modiolus capax* (Garza-Aguirre and Bückle-Ramírez 1989), *Chione californiensis* (García-Domínguez et al. 1993), *Glycymeris gigantea* (Villalejo-Fuerte et al. 1995), and *Laevicardium*

elatum (Villalejo-Fuerte et al. 1996a). Accordingly with Sastry (1970), although temperature affects reproduction, other environmental factors seem to interact with it in determining the pattern of annual gonad activity in a given geographical area. It is likely the variation in annual reproductive activity of a species will be the phenotypic response of a single genotype.

Food availability has been related to the timing of reproduction in some bivalves (Sastry 1979, Bayne and Newell 1983, MacDonald and Thompson 1985, Jaramillo et al. 1993). For example, in *Chlamys amandi*, the spawning time seemed to be related to food availability (Jaramillo et al. 1993); whereas, *Hinnites giganteus* showed no correlation between food availability and spawning (Malachowski 1988). The reproductive cycle of *P. multicostata* at Isla Espíritu Santo did not exhibit a clear relation with food availability, because the spawning extends all year, independent of food availability, expressed as photosynthetic pigment concentration. In *Pinctada mazatlanica* from the same location, maximum spawning did not coincide with maximum food availability (García-Domínguez et al. 1996).

Although, spawning of *P. multicostata* did not seem to be related to temperature or food availability, its spawning may be triggered by other factors, such as day length, a particular lunar

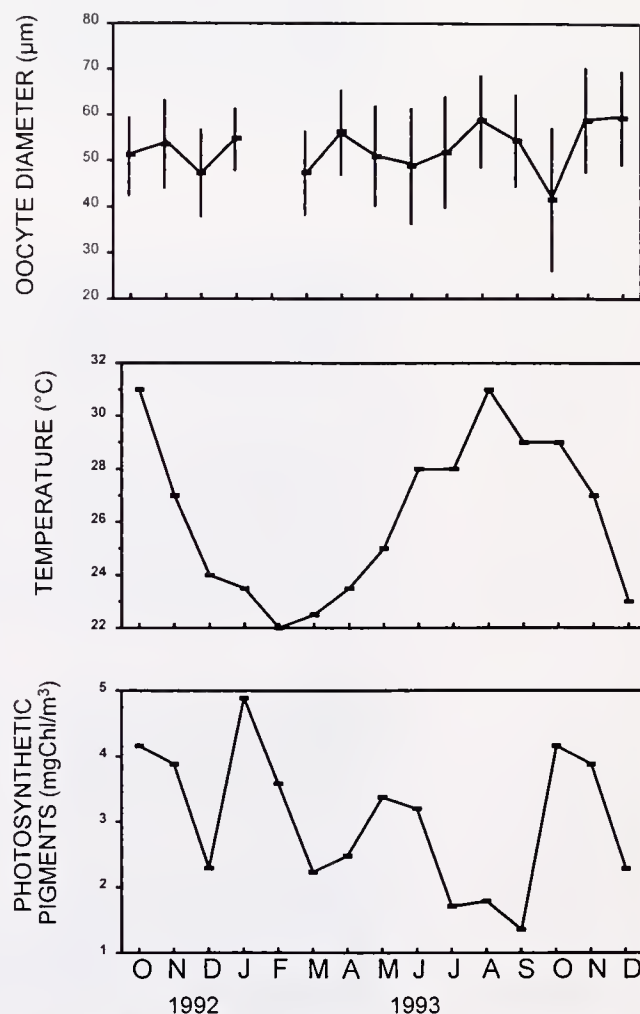


Figure 5. Mean oocyte diameters of *P. multicostata* (bars = standard deviation), water temperature, and photosynthetic pigment concentration from Isla Espíritu Santo, BCS, Mexico.

phase, salinity fluctuations, tidal cycle, or a combination of several of these. Unfortunately, in this study did not consider these other environmental factors.

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THE EVOLUTION OF A MUNICIPAL QUAHOG (*HARDCLAM*), *MERCENARIA MERCENARIA* MANAGEMENT PROGRAM, A 20-YEAR HISTORY: 1975–1995

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ABSTRACT Local municipal control of shellfisheries has been in existence in Massachusetts since 1942. The shellfish management programs of Orleans, MA, a town located at the elbow of Cape Cod, have evolved from transplants of native stock to use of hatchery-raised seed. For the period 1975 to 1995, the town utilized several forms of nursery culture in three separate estuaries including bottom culture, raft culture, a municipal hatchery, a land-based upweller system, tidal upweller, and floating trays. Financial constraints, as well as political and social perceptions determined the extent of the program at any given time. Management decisions were based primarily on survival of the seed rather than such factors as fast growth. The most successful method was a land-based upweller system with which we raised 1 million seed per year at 95% survival that were transplanted throughout the town. Survival in the field was directly related to water temperature at time of planting, which was most successful when water temperature was about 45°F (7°C).

KEY WORDS: Quahog, hard clam seed, *Mercenaria mercenaria*, shellfish management, nursery culture, aquaculture

INTRODUCTION

Efforts to observe growth of northern quahogs, also known as hard clams (*Mercenaria mercenaria* Linne) or to increase the natural production have been attempted since the early part of this century. Belding (1912) described both bottom culture and off-bottom methods used in four separate locations in Massachusetts. Haskin (1952), Carriker (1959), and Carriker (1961) added data to our understanding of environmental aspects of water and sediment that increase quahog growth, survival, and abundance.

Larval culture began with Wells, who cultured five molluscan species through metamorphosis and patented his methods in 1933 (Manzi and Castagna 1989). Loosanoff and his colleagues at the Bureau of Commercial Fisheries Laboratory in Milford, Connecticut (now National Marine Fisheries Service Laboratory) are credited with numerous developments in rearing of bivalve mollusks from spawning through the juvenile stage (Loosanoff and Davis 1950). (Loosanoff and Davis 1951), and (Loosanoff and Davis 1963). Once larvae and juveniles were readily available, knowledge of quahog culture expanded enormously (Judson et al. 1977, Manzi and Castagna 1989, Rice, 1992).

As a direct result of the culture efforts, entrepreneurs developed commercial hatcheries and field grow-out businesses. Towns in Massachusetts that manage their own shellfish resources purchased seed from hatcheries. Municipal shellfish programs, such as those on Cape Cod and Martha's Vineyard, Massachusetts, utilized this source of hatchery seed to develop their propagation schemes.

George Souza, Shellfish Constable of Falmouth, MA was the first shellfish officer to take advantage of these seed. Working with staff biologists from the Massachusetts Division of Marine Fisheries, in 1972, he developed an off-bottom culture system for raising small seed to be planted in the wild. By 1977, eight Cape Cod towns: Bourne, Barnstable, Chatham, Dennis, Eastham, Orleans, Wellfleet, and Yarmouth and the Martha's Vineyard Shellfish Group followed his example. Information was shared among the towns through workshops sponsored by the Division of Marine Fisheries. The focus of this paper is to describe the municipal propagation efforts that took place in Orleans from 1975 to 1997.

Municipal Management

Massachusetts is one of the few states where municipal management of shellfish resources is the norm. Towns are encouraged to promote and protect those resources under broad guidelines set by the Commonwealth. Towns appoint a shellfish constable and staff, promulgate and enforce local regulations, issue harvest licenses, determine harvesting techniques, determine areas suitable for harvest, conduct experiments in propagation, and issue licenses for private aquaculture. The state sets size limits, determines the species to be regulated, administers all aspects of contaminated areas, sets maximum fees for shellfish leases, and surveys potential lease sites for suitability and compliance with state law.

Belding (1912) examined many aspects of quahog life history and concluded that, without culturing this animal, the fishery would collapse.

Over half a century later, in 1975, the town of Orleans purchased 10,000 seed quahogs from Coastal Zone Resources, a hatchery in North Carolina to begin propagation experiments. Our primary objectives were to determine:

1. if hatchery-raised seed would survive transplant into a protected environment;
2. if seed would survive and grow under varying environmental conditions;
3. limiting factors of seed growth, including density;
4. if seed quahogs would survive in areas devoid of native stock; and
5. predators and estimate loss.

Initial field trials were somewhat successful, and we continued and expanded the program throughout the next 14 years. During this period, we developed a number of secondary objectives. These were:

6. to monitor transplants and determine survival;
7. to find economical methods for growing seed;
8. to produce as many quahogs as funds would allow; and
9. to increase quahog stocks in areas of Pleasant Bay that had been marginally productive for 20 or more years.

The program is presented chronologically. This emphasizes the transition as we built on success, learned from mistakes, and experimented with alternatives. Each phase of the program was gov-

erned by logic, financial constraints, and management principles. Much of the discussion is based on the observations of the author, only some of which were quantified. The program is divided into eight separate sections as follows:

1. bottom culture;
2. raft (off-bottom) culture;
3. hatchery;
4. upweller;
5. transplants to the natural environment;
6. changes in direction;
7. private aquaculture; and
8. budget constraints.

Methods for evaluation varied according to the specific propagation culture used at the time. Volumetric counts were made for each shipment of seed delivered. Square foot samples were dug with bottom culture, and survival and growth were estimated; samples of seed (approximately 100 randomly gathered) from the rafts were measured for growth, and the entire harvest was volumetrically counted to arrive at survival percentages; and volumetric counts were made from the upweller technique. Lack of technical staff and a desire to transplant as soon as possible from harvest limited our ability for more detailed statistical analysis. Seed were measured in metric units; construction details are dealt with in standard units.

Study Area

Orleans is located at the "elbow" of Cape Cod, MA (Figs. 1 and 2). It has three separate embayments within its boundaries: Cape Cod Bay, Nauset/Town Cove, and Pleasant Bay. Although different from one another, all support, or historically have supported, natural populations of quahogs.

The Orleans municipal jurisdiction in Cape Cod Bay extends six miles (9.7 km) from shore and includes approximately 1.5 miles (2.4 km) of intertidal sand flats that are nonproductive for

quahogs because of harsh environmental conditions, including heavy ice buildup and shifting sands. The depth offshore ranges from 0 to 25+ feet (7.5 m). Abundant beds of quahogs have historically been found in much of the deeper waters of the bay and are still harvested commercially by a few medium-sized (35 ft, 10.5 m) draggers.

The Nauset estuary (2,333 acre) is a very productive area (Roman et al. 1989), shared by the towns of Orleans and Eastham and protected from the Atlantic Ocean by a migrating barrier beach. Approximately 1,150 acres are in Orleans, but Orleans and Eastham share a reciprocal fishing agreement. Quahogs are found along the edge of the Town Cove, in eelgrass (*Zostera marina*), sand/silt/mud combinations, and along the steep gradient that leads to deeper water (6–18 ft; 2–6 m), as well as many parts of Nauset Harbor. They are harvested by hand scratchers or bull rakes.

Pleasant Bay, a larger estuary, is shared with Chatham, Harwich, and Brewster (no reciprocal fishing agreements); 3,500 acres are within the boundary of Orleans. A migrating barrier beach extending approximately 12 miles (19 km) to Chatham Inlet protects the bay from the Atlantic Ocean. Of the three estuaries, Pleasant Bay has had the least stable quahog population (based on landings). This may result from the barrier beach dynamics. A large set of seed (less than 2" legal size, [50 mm] in longest diameter) was discovered in Pleasant Bay in the late 1950s. Gates (1964) conducted a survey of Big Bay, making 33 samples in 27 acres, and found up to 180/0.3 m² with an average density of about 50/0.3 m². He estimated a standing crop of 60.7 million animals worth \$1.026 million at that time, providing a steady supply of shellfish and employment. They were well known in the marketplace for their long shelf life and could be easily identified as originating from Pleasant Bay, because their growth rings were almost indistinguishable from one another. The population lasted until the early 1970s, at which time, quahogs became rare in the bay, with no recurring set.

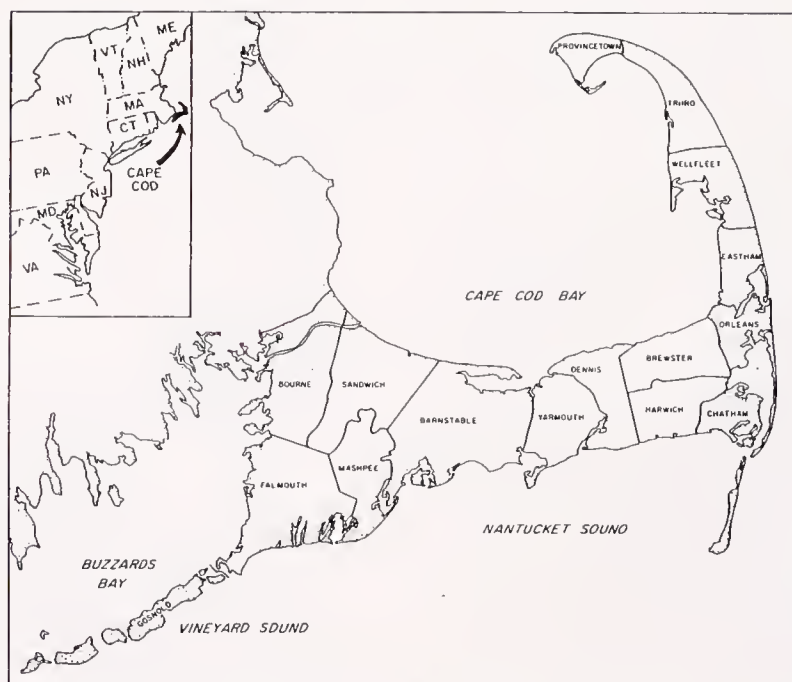


Figure 1. Locus map of area. Cape Cod is the easternmost land mass of Massachusetts. The Town of Orleans is situated at the "elbow" of Cape Cod.

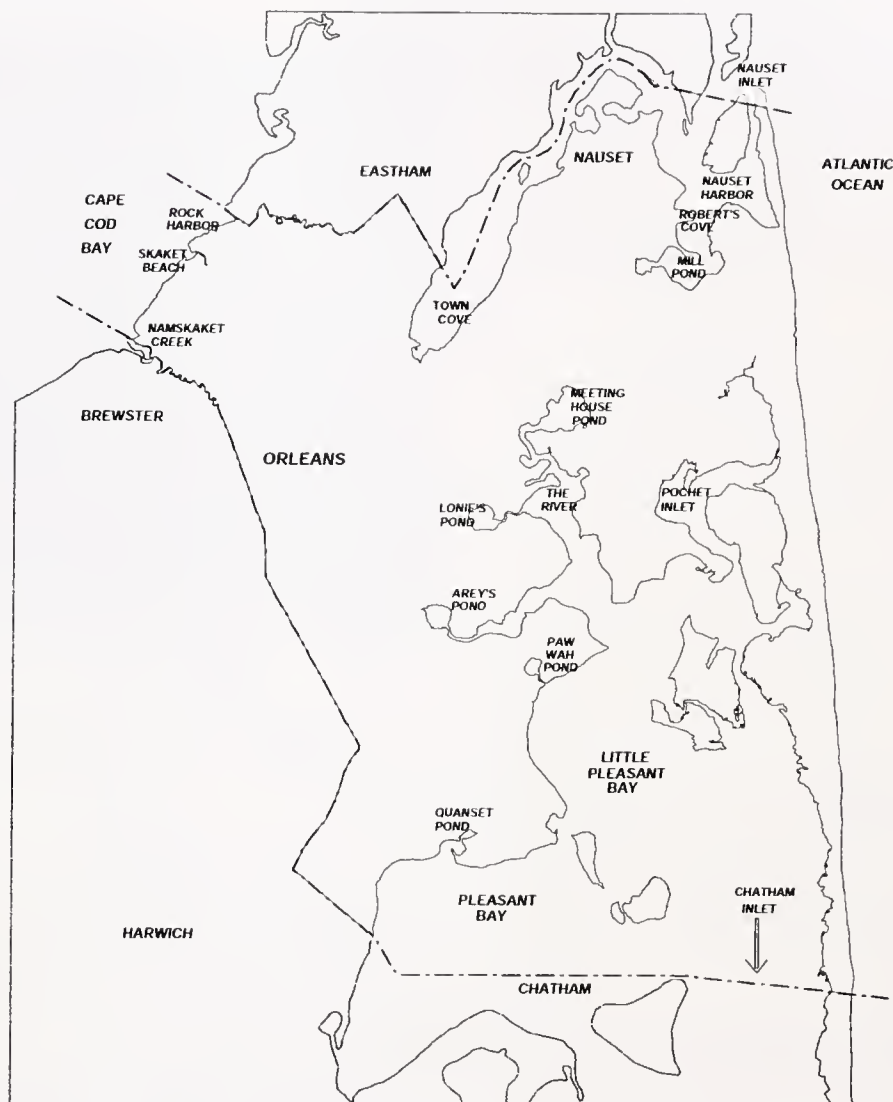


Figure 2. Map of Orleans, Cape Cod Bay, Nauset/Town Cove and Pleasant Bay.

BOTTOM CULTURE

We used bottom culture of seed for 3 years at a total of 20 locations (Fig. 3). Three different enclosure designs were deployed in all three estuaries.

1975

We chose 10 separate locations, with varying sediment, current, and other environmental conditions, to plant the 10,000 seed quahogs, approximately 8-mm in size. Frames, 3' x 6' were constructed of 1" x 2" "strapping" on end to which a 3/16" mesh netting was stapled. Each intertidal area was raked to loosen the substrate and remove any visible predators, and the seed was broadcast within the raked area (Fig. 4). The frame was placed over the seed with the edges buried and was secured by stakes at the corners and attached to the frames.

Results

Table 1 provides details of the experimental planting for 1975. We considered survival above 90% to be excellent, 75 to 90% very

good, 50 to 75% good, 25 to 50% poor, and less than 25% very poor.

With the exception of the frame at Namequoit Point, which was lost in a storm, survival in the summer was excellent. Quahogs grew from 8 mm in July to 11 to 18 mm in October, depending, in part, on location. The seed at Snow Shore exhibited the least growth. Native stock in the general vicinity also had slow growth (and unusually thick shells, often associated with slow growth).

Survival after the winter was excellent in two locations, very good at two, and disappointing in five locations where the survival was poor or very poor, despite an unusually mild winter. Most of the stock at Snow Shore and Meetinghouse River and half from Asa's Landing and the Yacht Club had died. The frame at Skaket had disappeared. We observed no correlation between sediment type or specific estuary and loss. In the spring at Asa's Landing, we observed live animals interspersed with empty shells in the top 25 mm of substrate. Below them, at around 33 mm, patches of black empty shells were found in black sulfurous smelling sand, adjacent to live seed in nonblack sediment. This initial observation was seen throughout the years in relation to the phenomenon known as "winter kill."

ORLEANS SEED BOTTOM CULTURE
QUAHOGS

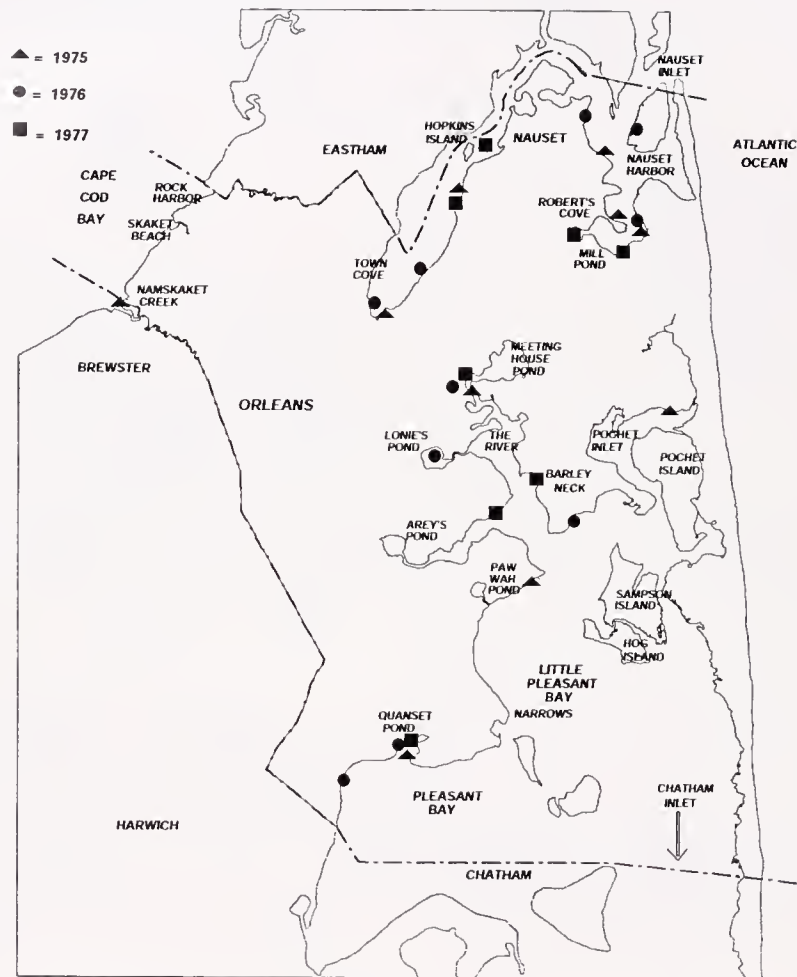


Figure 3. Bottom culture experiments were conducted in 20 separate locations in all three estuaries over a 3-year period.

1976

In 1976, we expanded the program and purchased 280,000 seed from two sources: 150,000 from Coastal Zone Resources and 130,000 from ARC (Aquacultural Resources Corp.), a local hatchery. Nearly 150,000 were used for intertidal bottom culture. A portion, 30,000, were large enough to plant directly into the natural environment, and the remaining 100,000 were grown in floating rafts (see next section).

The bottom culture enclosures were larger, 9' x 6' constructed of 1" x 6" wooden boards. Each box was divided longitudinally for strength. We added netting to the bottom to enhance recovery, because a few quahogs had been found outside the frames in 1975. We also built smaller boxes, 4' x 3', installed at seven additional sites with 5,000 ARC seed at each site.

Two boxes were installed side by side at three locations: Doane Way, Yacht Club, and Lonnie's Pond. One box was planted with ARC stock and the other with CZR stock in equal numbers to see if there was a variation in growth or survival depending on the source.

Results

Table 2 provides details of the plantings for 1976.

1. The ARC stock was smaller at the beginning of the season

(6–8 mm) and delivered 2 weeks later than the CZR stock (8–10 mm); 25,000 seed from ARC was delivered in mid-July (8–10 mm).

2. At Meetinghouse River and Quanset Pond, seed grew faster than those at other sites, 6 to 8 mm when planted to 19 mm in October.
3. In the side-by-side experiment with equal densities, the CZR stock, beginning at 8 to 10 mm grew to an average of 15 mm (average 6 mm of growth), and the ARC stock beginning at 6 to 8 mm grew to an average of 11.5 mm (average 4.5 mm of growth). The boxes at Lonnie's Pond could not be adequately evaluated because of the time difference of planting.
4. The average rate of growth at the other sites, 6.3 mm, did not seem to be dependent on the source of seed.
5. In the smaller boxes that survived, the lower density seemed to have a positive affect on growth.
6. The type of substrate did not seem to affect growth.
7. Where the boxes were tight enough to exclude predators, summer survival was excellent. Exceptions at Tonset, Pleasant Bay, and Barley Neck where boxes were lost, the loss was caused by boats, human interference, and storms.
8. Winter survival at Quanset Pond and Mill Pond was >80%.



Figure 4. Ten intertidal locations chosen in 1975, were raked to loosen the substrate and remove any visible predators. About 1,000 seed quahogs (*Mercenaria mercenaria*) were broadcast within the raked area and a frame, 3' x 6' to which a 3/16" mesh netting was stapled, was placed over the seed and staked in place. Edges were then backfilled.

and >50% survived in Meetinghouse River and Jack Knife Cove boxes, but survival was very poor at the remainder of sites.

The winter of 1976 to 1977 was exceptionally severe. Some bodies of water were frozen over for 14 consecutive weeks, and most of the boxes were severely damaged or destroyed. As an experiment, we had retained some covers and removed others before winter's onset. The boxes without covers sustained less winter damage than those with covers. A covered box at Doane Way was lifted out of the bottom leaving only the bottom netting, and a covered box at Meetinghouse River was physically moved 3.3 m. The covered box at Quanset Pond sustained no damage, but it had been installed on a fairly steep slope, which may have saved it from ice damage.

1977

Because of the damage to intertidal boxes from the severe previous winter, we modified the box design again for subtidal culture. Boxes were identical except that bottom netting was deleted and covers that could be handled under water were constructed. The covers were made of two layers of 1" x 3" strapping laid flat with netting securely sandwiched between. These were attached to the boxes by rubber hinges and held down by clasps. Boxes were trenched at ground surface but rocks and clay deposits sometimes prevented complete trenching. In these cases, boxes were above ground and had to be filled around the base.

Results

Table 3 provides details of the plantings for 1977. Quanset Pond, Meetinghouse River, and Jack Knife Cove showed continued excellent summer survival and good growth. Except for the one at Meetinghouse River, they also showed very good winter survival. Two anomalies occurred this year. First, large 14 to 16-

mm CZR stock was purchased and planted in May, but in three locations, Mill Pond, Little Mill Pond and Hopkins Island Channel, it only grew to 17 or 18 mm by November. Second, at Lonnie's Pond, stock that was 8 to 12 mm in May only grew to 13 mm. We do not know the reasons for such limited growth except that in Little Mill Pond and Lonnie's, the shells began to erode by the end of the summer.

The hinged cover design did not exclude predators, and above-ground boxes led to poor water exchange within the boxes, serious predation, and over-all poor results occurred.

Discussion

Seed quahogs were planted at 20 separate locations over a 3-year period with three different enclosure designs. Two sources of seed were used, CZR from North Carolina and ARC from Massachusetts. Size at planting ranged from 6 to 16 mm. Density ranged from 55 to 230/0.3 m², with one box at 456/0.3 m². Substrate ranged from coarse sand and cobbles to clay/mud, with the majority being a mixture of sand and silt. Most locations had either natural quahogs or soft-shell clams in the general vicinity, but a few locations had neither. Except for storm damage and human interference, most animals survived well over the course of the summer. Survival after the winter varied from a high of 50 to 90% in some areas to 0 to 10% in others. Some of this was caused by the severity of the winter.

Summer survival of ≤50%, was generally attributable to predation. Inadequate installation of the boxes resulted in washouts around the edges leaving holes under the boxes. Covers that were not tight allowed invertebrates to lodge between the box and cover. In both cases, predators entered and consumed the quahogs. We suspect that predators also may have entered the boxes as larvae and grew within the enclosure based on the time of year of deployment.

The bottom culture project as described employed many variables, and there were no "controls" as part of the program. This is because the primary objective was to increase quahog stock throughout the town.

Winter conditions proved to be the greatest obstacle to intertidal quahog culture that relies on structures and netting. Removing top netting just prior to a freeze lessened the physical damage but did not assure seed survival. Seed from North Carolina survived equally as well as local stock. Cost of materials was about \$1,000 for the 3 years of bottom culture and cost of seed was about \$4,000.

RAFT CULTURE

Raft culture methods were used in 1976 to 1987. Although the methods were essentially the same for the entire period, the amount planted, source of supply, and amount of growth varied during this time span.

Prior to 1975, George Souza, Falmouth Shellfish Constable and Arnold Carr, Massachusetts Division of Marine Fisheries, experimented with floating trays to grow quahog seed. They found that trays without sediment did not work well. Souza designed successful floating sand box rafts in Falmouth. In 1976, we decided to build rafts, but Souza's design was unwieldy for Orleans' departmental equipment to handle. We modified his design to our needs (Fig. 5).

The inside of the 9' x 6' wooden frame was covered with 1" vinyl-coated galvanized wire, 1/8" plastic netting, and window

TABLE 1.

Ten sites in three estuaries with varying environmental conditions chosen for initial intertidal bottom culture nursery plantings in 1975.

1975 Bottom Culture										
Location	Estuary	Source	Size	Date Planted	Density Ft 2	Substrate	Natural Clams/Q	Survival Summer	Size (MM) Oct.	Survival Spring '76
Skaket	CCB	CZR*	8-10 mm	July 75	55	Sand	None	90%	15	LOST
Doane Way	N	CZR	8-10 mm	July 75	55	Coarse sand/silt	SS/Q	95%	17	80%
Robert's Cove	N	CZR	8-10 mm	July 75	55	Silt/mud/sand	SS	95%	13.5	90%
Snow Shore	N	CZR	8-10 mm	July 75	55	Coarse sand	Q Near	75%	11	5%
Asa's Landing	N	CZR	8-10 mm	July 75	55	Sand	SS	95%	18	55%
Yacht Club	N	CZR	8-10 mm	July 75	55	Coarse sand	SS/Q	95%	18	50%
Meetinghouse River	PB	CZR	8-10 mm	July 75	55	Sand/silt	None	95%	18	10%
Namequoit Point	PB	CZR	8-10 mm	July 75	55	Coarse sand/grave	None	0%	N/A	0%
Quanset Pond	PB	CZR	8-10 mm	July 75	55	Coarse sand	Q Near	95%	17	90%
Pochet Landing	PB	CZR	8-10 mm	July 75	55	Med. fine sand	SS Near	95%	16	80%

CCB = Cape Cod Bay; N = Nauset; PPB = Pleasant Bay; SS = soft shelled clams (*Mya arenaria*); Q = quahogs (*Mercenaria mercenaria*); *Coastal Zone Resources.

screen material. Each raft was filled with at least 4" (100 mm) sand from an inland site. Styrofoam blocks were attached to two long sides. Two short sides were open to allow for a flow-through of water. Bright orange floatation blocks (9' x 1' x 1') were painted with green nonantifouling paint so the rafts would blend with the landscape. Each raft was moored by 5/8" galvanized chain to a 50 lb (23 kg) mushroom mooring on the short end to allow the raft to swing in the tide. Threaded rods, used to assemble the structure, held galvanized chicken wire to prevent bird predation.

The rafts were planted with 25,000 seed at a density of about 400/0.3 m². Two salt ponds were selected for deployment: Mill Pond (Nauset) and Lonnie's Pond (Pleasant Bay) (Fig. 3). Mill Pond was chosen, because it has native populations of shellfish, is protected from motor boat activity by a rock dam, and is protected from most winds except NE. A second raft was deployed in Little Mill Pond, a small shallow, <3' (<1 m) pond adjacent to Mill Pond

that is not productive for shellfish, but is secluded and protected from strong winds. Lonnie's Pond was chosen because of limited boating activity, protection from wind, and natural production of soft-shell clams. The water in Mill Pond was consistently 4°F (2.2°C), and the salinity was about 4‰ higher than Lonnie's Pond.

1976

The rafts were planted in May with 100,000 seed and remained in the water until October. They were emptied using a Venturi-type hydraulic pump with a 1/2" mesh hardware cloth basket attached. The mesh allowed the sand to wash through but retained the quahogs. Harvest mortality was negligible, except in cases where sand had not been carefully screened and contained large stones. Seed was counted volumetrically, and the quahogs were then transplanted to the natural environment (see separate section on transplants).

TABLE 2.

Twelve sites were chosen for intertidal bottom culture plantings in 1976.

1976 Bottom Culture											
Location	Estuary	Source	Size	Date Planted	Density Amount	Density Ft 2	Substrate	Natural SS Clams/Q	Survival Summer	Size (MM) Oct.	Survival Spring '76
Doane Way	N	CZR	8-10 mm	3 June	12,500	230	Sand/silt	SS/Q	90%	15	0%
Doane Way	N	ARC	6-8 mm	17 June	12,500	230	Sand/silt	SS/Q	85%	11.5	10%
Yacht Club	N	CZR	8-10 mm	3 June	12,500	230	Coarse sand	Q	98%	15	10%
Yacht Club	N	ARC	6-8 mm	17 June	12,500	230	Coarse sand	Q	98%	11.5	10%
Town Cove	N	ARC	6-8 mm	17 June	12,500	230	Coarse Sand	SS	98%	13	0%
Meetinghouse River	PB	ARC	6-8 mm	17 June	12,500	230	Sand/silt	None	98%	19	50%
Quanset Pond	PB	ARC	6-8 mm	17 June	12,500	230	Coarse sand	Q Near	98%	19	90%
Nauset Bar	N	ARC	6-8 mm	17 June	5,000	90	Sand	SS	90%	13	0%
Mill Pond	N	ARC	6-8 mm	17 June	5,000	90	Sand/silt	SS	98%	15	85%
Tonset	N	ARC	6-8 mm	17 June	5,000	90	Coarse sand	Q	0%	0	0%
P. Bay	PB	ARC	6-8 mm	17 June	5,000	90	Coarse sand	None	0%	0	0%
Jack Knife Cove	PB	ARC	6-8 mm	17 June	5,000	90	Sand/silt	None	98%	15	50%
Barley Neck	PB	ARC	6-8 mm	17 June	5,000	90	Coarse sand	SS	0%	0	0%
Lonnie's Pond	PB	ARC	6-8 mm	17 June	5,000	90	Silt/sand	None	98%	17	0%
Lonnie's Pond	PB	CZR	8-10 mm	15 July	25,000	465	Silt/sand/clay	None	98%	17	0%

N = Nauset; PB = Pleasant Bay; SS = soft shelled clams (*Mya arenaria*); Q = quahogs (*Mercenaria mercenaria*); CZR = Coastal Zone Resources and ARC = Aquacultural Resources Corp. as sources of seed.

TABLE 3.
Eight sites were selected for sublidal bottom culture plantings for 1977.

1977 Bottom Culture												
Location	Estuary	Source	Size	Date	Amount Planted	Boxes	Density	Substrate Ft 2	Natural SS Clams/Q	Survival Summer	Size (mm) Oct.	Survival Spring '76
Jack Knife Cove	PB	CZR	8-12 mm	18 May	27,000	3	165	Sand/silt	None	90-95%	20	75%
Meetinghouse River	PB	CZR	8-12 mm	18 May	18,000	2	165	Silt/sand	None	90-95%	19	0%
Lonnie's Pond	PB	CZR	8-12 mm	18 May	18,000	2	165	Sand/clay	SS	80%	13	20%
Quanset Pond	PB	CZR	8-12 mm	18 May	18,000	2	165	Silt/sand	Q Near	90-95%	18	75%
Mill Pond	N	CZR	14-16 m	26 May	9,500	1	90	Coarse sand/Rocks	SS	15-20%	17	5%
L. Mill Pond	N	CZR	14-16 m	26 May	9,500	2	90	Silt/sand	SS Near	50%	17	10%
Hopkins Is. Chan.	N	CZR	14-16 m	26 May	4,700	1	90	Sand/rocks	Q/SS Near	40%	18	5%
Asa's Landing	N	CZR	14-16 m	26 May	19,000	2	175	Silt/sand	SS	50%	21	10%

N = Nauset. PB = Pleasant Bay; SS = soft shelled clams (*Mya arenaria*); Q = quahogs (*Mercenaria mercenaria*). All stock originated from Coastal Zone Resources (CZR).

Results

Table 4 summarizes the raft culture program in Orleans from 1976 to 1980.

1976 Results

There was negligible summer mortality, no evidence of predation, and excellent growth. Fouling was a problem, especially in Lonnie's Pond, where seaweeds attached to the wood or appeared as clumps within the rafts. Seaweed became dense enough to cause smothering if the rafts were improperly maintained. Barnacles, tunicates, and bryozoans were the principle animal fouling communities, but we have no evidence that they adversely affected the seed growth or survival. The rafts in Mill Pond had less seaweed or organism fouling, but blue mussels (*Mytilus edulis* Linne) set prolifically on the undersides of the rafts. This added substantial weight.



Figure 5. Rafts were deployed in Mill Pond (Nauset) and Lonnie's Pond (Pleasant Bay). The rafts were planted with 25,000 seed at a density of about 400/0.3m².

Birds, principally gulls and ducks, sat on the floats and left their droppings, but did not predate them. The mesh holding the sand in place was too fine to allow for organic waste removal. Black sand built up by the end of the season but had no apparent negative effect on the animals. In Lonnie's, animals that were 8 to 10 mm when planted in May were 25 to 30 mm by October. In early September, many of the animals were on top of the sand because of crowding, but growth and/or survival did not seem to be affected. We harvested these rafts by the end of September to prevent potential crowding-related problems. The seed planted on the Mill Pond rafts exhibited a slower growth of 20 to 25 mm. This was despite their having been planted a week earlier than the seed in Lonnie's and harvested almost a month later.

At the end of the season, those quahogs that were less than 15 mm were put back on the raft to hold over for the winter. As previously noted, the winter of 1976 to 1977 was unusually severe. The rafts were frozen in ice for 12 to 14 consecutive weeks. We estimated 20% mortality when we could get out to the rafts and an additional 20% when the water temperature increased to about 50°F. Black sand was found throughout the raft (winter kill).

1977

In addition to rafts in Lonnie's Pond and Mill Pond, a raft placed in Quanset Pond (PB), was painted with antifouling paint and planted with 20,000 seed, as an additional experiment. The paint did not seem to affect growth or survival.

1977 Results

See Table 4 for details on raft culture for 1978. A strong NE storm damaged the rafts in the Mill Pond and 10 to 20% of the seed washed out of the rafts.

1978

We ordered 400,000 seed quahogs but only received 17,000. Coastal Zone Resources went out of business, and ARC could not supply our needs. A third hatchery, Shellfish Inc. (SI) in Sayville, NY shipped 17,000 in August. They did not grow large enough to be transplanted and were overwintered on the rafts. Approximately 60% survived.

1979

We ordered 300,000 and received 275,000 (see Table 4).

TABLE 4.

Summary of raft culture in Orleans from 1976 through 1980 at two sites; rafts were not deployed in 1978 because of unavailability of seed.

1976 Raft Culture										
Location	Estuary	Source	Amount	Rafts	Size	Date Planted	Density Ft ²	Survival Summer	Size Oct.	Survival Spring
Mill Pond	N	CZR	25,000	1	8–10 mm	20 May	400	95%	25–30	65%
Little Mill Pond	N	CZR	25,000	1	8–10 mm	20 May	400	95%	25–30	65%
Lonnie's Pond	PB	CZR	50,000	2	8–10 mm	26 May	400	95%	26–30	65%
1977 Raft Culture										
Mill Pond	N	CZR	60,000	2	8–12 mm	18 May	475	80%	25	N/A
Mill Pond	N	CZR	20,000	1	8–12 mm	26 May	315	80%		
Lonnie's Pond	PB	CZR	30,000	1	8–12 mm	18 May	475	95%	30	N/A
Lonnie's Pond	PB	CZR	50,000	2	8–16 mm	26 May	400	95%	30	N/A
Lonnie's Pond	PB	ARC	35,000	1	5–9 mm	21 July	180	95%	12–18 mm	N/A
Quanset Pond	PB	ARC	20,000	1	5–9 mm	21 July		95%	10–16 mm	N/A
1979 Raft Culture										
Mill Pond	N	ARC	100,000	3	5–6 mm	31 May	617	95%	15–25 mm	N/A
Lonnie's Pond	PB	ARC	100,000	3	5–6 mm	7 June	617	95	25 mm	N/A
Lonnie's Pond	PB	SI	75,000	1	2 mm	28 June	1400	40%	6–15 mm	N/A
1980 Raft Culture										
Lonnie's Pond	PB	BSP	100,000	3	8–19 mm	1 Apr	617	95%	25–30 mm	N/A
Lonnie's Pond	PB	ARC	100,000	4	5–6 mm	30 May	463	95%	18–30 mm	N/A
Lonnie's Pond	PB	ARC	100,000	4	5–6 mm	2 July	463	95%	18–30 mm	N/A

N = Nauset; PB = Pleasant Bay; CZR = Coastal Zone Resources; ARC = Aquacultural Resources Corporation; SI = Shellfish Incorporated; BSP = Bristol Shellfish Products.

1979 Results

The small size of the SI seed may have resulted in low survival (40%) in the raft by the end of the season. We observed animals being washed out with boat wakes and during high winds and believe the balance of seed was washed out. The remaining stock grew to 6 to 15 mm. The ARC stock growth was comparable to previous years, and survival was 95%.

1980

We bought 100,000 seed from a fourth hatchery, Bristol Shellfish Products (BSP) in Maine, which we planted very early, April 1. We purchased 200,000 from ARC and planted half on May 30 and half on July 2. All were planted in Lonnie's Pond rafts.

1980 Results

Fouling was extremely heavy. See Table 4 for growth and survival results.

Discussion

From 1976 to 1980, we used two ponds, four suppliers of seed, with size at planting ranging from 2 to 19 mm for the rafting program. Seed was planted as early as April 1 and as late as July 21, with optimal planting in May and June, when weather had stabilized. Predation was negligible. Summer survival was 95% or more, regardless of the density, except in three instances, where the shellfish were lost because of washout in storms. Seed, less than 5 mm at planting time, were too small for this system, because they were easily washed out by waves, including boat wakes.

The major advantages of the raft system was increased growth rates and ability to concentrate density of plantings. Most seed

reached the state-recommended plantable size of 25 mm by the fall, and thus overwintering problems associated with gear in the water could be avoided.

We continued to use the rafts through 1987. Lonnie's Pond had proved to be superior for growth and survival. Although we were concerned about putting all our seed in one location, we used only Lonnie's from 1980 to 1987. Growth was still good, and mortality was negligible. Fouling in 1980 was severe, and we had noticed that the water quality in Lonnie's was beginning to show signs of decline. Average salinity dropped by 1‰, water color was often tinged red (monospecific blooms of dinoflagellates), and the amount of floating seaweeds, both green filamentous (*Enteromorpha* sp.) and red (*Calithamnion*) increased dramatically. Taken together, these are signs of eutrophication. As the 1980s progressed, fouling increased. The Pleasant Bay circulation was affected by a southward migration of the protective barrier beach. This diminished the exchange of oceanic water that reduced the salinity. In 1987, a breach occurred in the barrier beach at Chatham, MA. This drastically changed the hydrodynamics of the bay, including a 1-ft (0.3 m) change in tidal amplitude. By that time, we had changed our nursery culture methods (see section on Hatchery/Lab below).

Mill Pond continued to have good water quality, but because of potential storm damage and slower growth, we were reluctant to use Mill Pond extensively.

The primary problem with the rafts was their size and amount of clean sand required. Lonnie's was small, and we had maximized the amount of area we felt should be devoted to this project. The pond was also used by recreational boaters, and the number of moorings in the pond was increasing each year. The rafts had been damaged through boating activity, and we feared vandalism or accidents if the number of rafts was increased.

During the course of the program, the seed cost increased sub-

stantially, and the availability in spring fluctuated greatly. The only way we could obtain the number of seed required was to purchase smaller animals; however, with seed less than 5 mm, losses in the raft system were severe. The Mill Pond was reasonable for growth and survival but was not secure from wind damage, and removing the annual mussel set was labor intensive.

The rafting method of nursery growout was more expensive and labor intensive than bottom culture, but, in Orleans, consistent high growth and survival were achieved in both Lonnie's Pond and Mill Pond. The rafts cost about \$250.00 each (1978) for materials, and they had a life span of at least 5 years. We preferred a density of about 450/0.3 m², but we planted as many as 30,000/raft, 555/0.3 m², without impeding growth or survival. The highest density bottom culture was close to 200/0.3 m². We did not determine the maximum density with these systems.

Clearly, growth rate was greater on the rafts than in the bottom boxes. The boxes were intertidal and, thus, exposed for several hours each tide, and the seed were unable to feed; whereas, on the rafts, the animals were submerged. The sand in the rafts seemed to have an affect on growth, because seed observed on rafts with sand washed out and density of animals sparse, the seed seemed to siphon less vigorously, and growth was less than seed observed in the sand.

After the winter of 1976 to 1977, we determined that seed should be planted in the fall, and we began to determine factors for best transplant survival. We realized, after several years, that if we planted the largest seed in September, it would survive fairly well, and if we planted the smallest seed in November, it also would survive well, but if we planted small seed in September, it would perish. Thus, we revised our thinking about the minimum size seed that could be successfully transplanted in the fall. (See section on transplants).

We could not increase the number of rafts in Lonnie's Pond because of other public uses. This limited the amount of seed that we could raise to 300,000. After 1980, we raised approximately 250,000 to 300,000 per year in rafts. The rafting program was halted in 1987.

HATCHERY/LAB

Site Selection

In 1979, the Shellfish Department office, a 16 ft × 24 ft (4.8 m × 7.2 m) building had to be relocated. The rapidly rising cost of seed in the late 1970s, unreliable seed supply from commercial hatcheries, and a requirement by Massachusetts Division of Marine Fisheries that seed could only be imported from hatcheries north of, but including, Long Island, prompted us to consider raising our own seed. Matthiessen and Toner (1966) developed hatchery methods for Martha's Vineyard, and we thought these would work in Orleans. Suitable waterfront land was limited to two parcels, neither of which was ideal or optimal. A small area on the shore of Lonnie's Pond was particularly appealing, but the site was located in a residential district. There was opposition from the neighbors who feared such potential negative aspects as unsightly gear, traffic congestion, and unknown problems.

The second site was on the shore of Town Cove. This included a public boat launching ramp in a business-zoned area. We moved the building to the Town Cove site (Fig. 6). In 1981, a bulkhead was constructed in front of the building, and a ramp and floats were added for public access to the water. This improvement increased the public usage of the landing. Site selection is critical to



Figure 6. A 16' × 24' (5.1 × 8 m) building was moved to the shores of Town Cove where a seawater system was installed. Within 2 years after moving the building, the town constructed a bulkhead that substantially enhanced the area for recreational boating. This led to incompatibility with the shellfish lab.

hatchery success, and our experience with site-related problems are illustrated.

Construction of a Seawater System

The budget of \$7,000 to equip the building for the project required installation of a single intake system using 1.5" pipe and a 1 hp swimming pool pump. Tanks were constructed of wood and fiberglassed. The tanks were 8 ft long and 4 ft wide, divided in the middle lengthwise. Valves and drains were installed in each compartment to allow for flexibility. Three tanks or six compartments were constructed so stock could be held and manipulated for various purposes.

When the bulkhead was being planned, it was recommended to install a dual intake system with pumps located at the bulkhead rather than in the building (90 ft [27 m] landward), but we were not permitted to do so. Instead, a single 2" (2.5 cm) pipe set within a 6" (1.2 cm) PVC pipe was buried under the parking area to guard against potential damage from automobile traffic and a larger pump (2 hp) was purchased. Although the larger pipe and pump were improvements, the system was difficult to maintain because of its inaccessibility. In addition, because we were pulling water rather than pushing it, air in the vacuum side of the pump was a constant problem.

HATCHERY METHODS

Algae Production

Glass bottles (12 narrow mouth 5 gallon [22 L] carboys and a number of 1 gallon [4.4 L]) were donated. Starter cultures of *Monochrysis*, *Isochrysis*, 3-H (*Thalassiosira*), and *Dunaliella* were obtained from the NOAA National Marine Fisheries Service laboratory in Milford Connecticut, Bigelow Laboratory for Ocean Sciences, and the Woods Hole Oceanographic Institute.

The cultures were grown in an illuminated, temperature controlled room (6' × 6' [1.8 m × 1.8 m]), with an air supply following the procedures detailed in Guillard (1974). Culture densities were estimated and recorded daily using standard methods. The general layout, equipment, and operation followed the design of algae rooms in similar sized hatcheries in Maine, (Smith, Heinig, pers.

comm.) We tried outdoor algae production, but the lack of a secure area resulted in accidents that were too numerous to overcome.

Quahog Culture

A hatchery in New England can operate either using natural ripened stock and commence spawning in June or condition the animals by slowly raising water temperature to commence spawning in January or February. Cost of winter spawning is high, because heated water is necessary to condition the quahogs. We chose using the natural spawning time of summer.

Quahogs used for spawning, came from $20 \pm$ feet (6 m) depth in Cape Cod Bay, native stock from the Town Cove, and transplanted hatchery stock from Pleasant Bay. Each animal was marked according to its origin.

Loosanoff and Davis (1950), (Loosanoff and Davis 1951), Loosanoff and Davis (1963), Loosanoff et al. (1953), and (Loosanoff and Davis 1955) developed hatchery procedures for bivalve mollusks. Other researchers, such as Castagna and Kraeuter (1977) and (Castagna and Kraeuter 1981) have refined the process to suit individual needs. We generally followed prescribed procedures for hatchery methods, substituting low-cost containers (see Table 5) whenever possible.

Castagna and Kraeuter (1981) used mass spawning methods, and researchers at the Milford Laboratory used individual spawning animals. Because we needed several spawns so that high larval or set mortalities would not preclude loss of the entire year's production, we chose the individual spawning method.

To induce spawning, two or three quahogs (from 37 to 100 mm) were placed in a Pyrex loaf pan with 1 micron filtered seawater (Fig. 7). Food was added when the quahogs began pumping. Hot tap water was added to the tank to raise the water temperature to a minimum of 27°C. If spawning did not occur, the tank water was cooled with ice and the process was repeated. Sperm suspension, either fresh or frozen, was added occasionally as rec-

ommended by Loosanoff and Davis (1963) and Castagna and Kraeuter (1981).

When spawning occurred, males and females were separated. Males were removed from the loaf pans after a few minutes, the shells were dried and marked as male. When the females were spent, they were marked correspondingly.

The eggs were sieved through a 50 micron sieve and placed in 30-gallon (32-liter) plastic trash cans as larval containers (Fig. 8). We followed the advice of other researchers regarding the viability of eggs as related to size (Castagna, Kraeuter, Gibbons, Rhodes, Goldberg, Chapman, Karney and others, pers. comms). We tried to ensure that several males were used and also several females to diversify the genetic pool.

Fertilized eggs were sieved after 48 hours. Low-cost sieves (see Table 5) were constructed from 5-gallon (22-L) plastic buckets with tight fitting lids donated by the food service industry. We removed the center of the lid leaving only the rim. We placed Nyltex netting tightly over the bucket top, replaced the lid rim, and sealed the inside edge with aquarium sealant. We cut off the bottom of the bucket so that when the bucket was inverted, the netting was on the bottom (Fig. 9).

Larvae were sieved every other day and were fed a diet of *Monochrysis*, *Isochrysis*, and *Dunaliella*, depending on the size and food availability. We did not try to increase production through the use of antibiotics (Hidu and Tubiash 1963, Castagna and Kraeuter 1981), because we did not know if there would be ramifications for future generations using antibiotics.

Our postset system is described in Table 5, setting system (1). The cascading trays (Fig. 9) were based on a design by Lind (Town of Eastham Dept. Natural Res. pers. comm.) in Eastham. This system was easier to handle than raceways described by Castagna and Kraeuter (1981) and Rhodes et al. (pers. comm.) However, our second type of system (Table 5) worked even better. While the veliger larvae were in the trash cans but ready to metamorphose, we added stacks of the trays (three trays per trash can)



Figure 7. Spawning in Pyrex loaf pans filled with seawater. Marks indicate estuary of origin and after spawning, individuals were also marked with gender symbols.



Figure 8. Eggs were placed in 30 gallon (32 liter) plastic trash cans at a density of 10 to 20 eggs/mL. Larvae were siphoned to sieves; the "dregs" remained on the bottom preventing batch contamination.



Figure 9. Inexpensive sieves were made from 5 gallon (19 liter) plastic buckets with tight fitting lids. Connector rings, (12" high) from the buckets ensured that no larvae were lost in the sieving process. Cascading glass "shelving" trays, (12" x 10" x 2") with attached mesh inside plastic "kitty litter trays" (15" x 12" x 3"), constructed for postset juveniles.

lined with appropriate sized mesh (Fig. 10). As metamorphosis occurred, the animals set on the mesh as well as on the bottom. Animals that set on the bottom were moved to the trays and kept in the trash cans until they were large enough to go into upwellers. We treated these postset animals as larvae until transferral.

UPWELLER TECHNOLOGY

Bayes, (1981) described a method for using "forced plankton-rich seawater up through a partially fluidized bed of filter feeding molluscs," which became known as the "upweller technique." The upweller technique had the potential of increasing the capacity at our site, and we developed a design based on standard fish totes (80 cm x 45 cm x 29 cm) placed in the tanks.

Figure 11 shows our upweller system. Each tote had two chambers (often referred to as "silos") made from the same type of 5-gallon plastic buckets as our sieves for a total of 36 silos. The bottom 3 inches of each bucket was cut off and the solid bottom was removed from that piece, resulting in a ring. Holes drilled in the ring (resembling a work ring) allowed water to flow up through the chambers placed on the ring. Mesh size was increased as the seed grew.

The system allowed us to eliminate the hatchery and purchase small, low-cost seed (1–2 mm) from commercial hatcheries. We purchased a million seed that were equally distributed among the silos. Silos were added as the seed grew, ending with a density of about 25,000/silo.

The chambers were rinsed with freshwater daily to remove waste products, and every other day, the chambers were rinsed, seed were removed, and the silo was washed with soap and water. Depending upon the biofouling severity, the chambers were cleaned with bleach and rinsed thoroughly. The same procedure

was used for the totes. The intake pipe was back flushed weekly with freshwater.

Food for the seed in the upwellers was primarily natural plankton; however, we supplemented this food with approximately 1 liter/tote of cultured algae per day. The water was shut off during feeding (just after cleaning in an attempt to maximize the benefit), and once the seed cleared the algae from the water, the flow was resumed.

On July 31, 1985, our technician noticed an oil slick next to the dock, within 100 ft (33 m) of our intake pipe. The slick was traced to a trash compactor behind a large supermarket and a trail of oil from the compactor lead to a lagoon with a direct discharge to Town Cove. Within 10 days, 90% of the seed quahogs were dead.

The small seed (2–6 mm) did not have enough tissue remaining to perform a bioassay (Tripp, pers. comm.). After a literature search and assistance from Dr. Judith (Capuzzo) MacDowall, we assembled enough information for the town to sue the supermarket. We claimed loss of the seed cost, the wholesale market value of the seed at maturity at 60% survival, and the cost of the equipment.

In 1987, we were awarded an out-of-court settlement of \$86,000 for the oil spill. A portion of the funds were used to install a full dual-intake system with new, larger (5 hp) pumps. We re-designed our tanks to a total of 48 individual chambers in which 1.0 million seed were grown, allowing us to decrease the density to 20,000/silo.

Discussion: Hatchery and Upwellers

We spent 4 years learning and perfecting methodologies for both algae and bivalve culture with our limited system. By 1984, we had over 150,000 seed that reached 3.0 mm by late August; however, when hatchery production did not approach expectations of millions of seed, we were required to abandon the building as a hatchery.

Seed in all postset systems during late July and early August seemed to be stressed. Survival during this stage was difficult, especially with the constant threat and reality of gas bubble disease resulting from the intake pipe design. Water temperature was high; 80 to 82°F (27–28°C) was not uncommon; oxygen was low; plankton in the seawater was predominantly dinoflagellates (a poor food); and any mesh smaller than window screen trapped air under the netting. Work by Anderson (1978) and Anderson (1979) on the local red tide organism *Gonyaulax tamarensis* (now *Alexandrium tamarensis*) suggested that *M. mercenaria* do not actively feed when *A. tamarensis* is in the water column. We suspect that other dinoflagellates may have the same effect. We aerated the tanks and added our own food as a supplement. Adding the food after cleaning seemed to be effective.

When we changed to upwellers, we were able to culture the 1 to 2 mm seed up to 12 to 18 mm by the fall. In 1986, 1987, 1988, and 1989, we raised a million seed per year, over 95% of which were transplanted to the natural environment.

Although the site was not conducive for a shellfish nursery, it had a major advantage over the Lonnie's Pond site—visibility. With a town landing, a marina, a large sporting-goods store, and a restaurant/inn all adjacent to the lab, it was located in a very public area. The lab hosted between 250 to 400 visits per year between June 15 and October 15, allowing us the opportunity to provide education to tourists and residents alike regarding the operation and general marine related topics. Table 6 summarizes hatchery/onshore nursery problems encountered and benefits to the town.

TABLE 5.

Summary of onshore hatchery/nursery methods including larval and postset containers, sieves and upwellers; sieves and upwellers were same basic design (see text).

System	Material	Shape	Size	Mesh	Density	Number
Larval containers	Plastic	Round	30 Gal. (132 L)	N/A	0.5–1.0 Million	8
Sieves	Plastic	Round	5 Gal. (22 L)	Variable	N/A	
Postset (1)	Glass with mesh attached	Square	1' × 1' (300 mm × 300 mm)	225 m	75–100,000/Tray	16
	Plastic	Rectangular	1' × 1.5' (30 cm × 45 cm)			16
Postset (2)	Plastic setting trays	Round with 4 compartments	3' (0.9 m) Diameter	Variable	0.5 Million	3/Trashcan
Upwellers	Plastic	Round	5 Gal. (22 L)	Variable	125,000–20,000 size dependent	12–48

TRANSPLANTS TO THE NATURAL ENVIRONMENT

Stock raised in intertidal bottom culture boxes remained in the general vicinity. Some were transplanted to reduce the density and some were planted in more subtidal locations. Stock grown on the rafts and in upwellers at the lab. was transplanted to many locations within the Town (Fig. 12).

The transplants were the final program stage. We needed to determine if planted, unprotected seed would survive in the wild, because the program goal was to augment native stock. We could not afford to protect the seed for a second year while maintaining seed production. Because this was a municipal program, survival was of paramount importance; whereas, both rapid growth rate and survival are important foci for private enterprise. Survival experiments by Flagg (1981), Castagna and Kraeuter (1977), (Kraeuter 1981), Menzel (1976), Carriker (1959), (Carriker 1961), and many others suggested that survival of seed mollusks was related to density and size.

Seed from raft culture attained a transplant size of 20 to 30 mm. Seed planted in the bottom boxes and those grown in the lab were

much smaller, averaging 12 to 18 mm at transplant time. Massachusetts Division of Marine Fisheries biologists Carr and Hickey, (pers. comm.) and McKenzie (1977) suggested that 25 mm was the minimum size needed for a high percentage of survival in the field. Because our seed did not always reach 20 mm, we needed to learn if a smaller size would survive in the field and the requirements for survival of small seed.

To distinguish propagation program stock from wild stock, we purchased *M. mercenaria (notata)* whenever possible. This stock had been bred with a genetic tracer of brown zigzag marks or stripes on the shell that remained throughout the animal's life. Approximately 50 to 60% of our quahogs exhibited the markings.

Method of Transplant

We broadcast quahogs over a wide geographic area. The bottom culture experiments and the literature suggested that dense plantings were highly preyed upon; whereas, predation might be



Figure 10. Mesh liners within compartments of plastic tiered setting trays placed in the trash cans as an alternative setting and postset system.



Figure 11. Upweller system of common plastic fish totes with bucket "silos." Constructed in the same manner as the sieves, each silo was placed on a bucket ring that had holes drilled (resembling a wok ring) to allow water to flow through the ring and up through the silo. Water pumped into the fish totes, flowed through the silos, and was discharged.

TABLE 6.
Summary of hatchery/onshore nursery problems and benefits in Orleans.

Problem					
Funding	Site Selection	Intake	Food Supply	Spawning	Postset
Insufficient	Construction of bulkhead:	Single pipe	Inadequate	Natural spawning time	Env. conditions July/Aug.
Inconsistent	Intake damage	Inaccessibility	No outdoor production		
Jury-rig	Commercial area	Pulled vs. pushed water	Lack of security	Individual spawning	High water temp.
Inadequate equipment	Marina				Low DO
	Highly used town landing	Pump size			Poor natural food
	Commercial businesses	Impractical to clean			Uneven food distribution
	Oil spill	Gas bubble disease			
	Loss of 1985 season	Pumps too small			Air bubbles under mesh
	Lack of evidence				Insufficient water flow
					Inexplicable mortalities
Benefit					
		Visibility	Upwellers		
		250–400 visitors per year	Program expansion to one million seed/year		
		Public education regarding shellfish life history and ecology	with high survival		
		Focal point and pride of town	No winter operation		

Insufficient funding and site selection were primary factors in degree of operational success but public nature of site raised public perception of program through interaction with culturists.

less at lower density. Because survival was the most important result, spreading seed over wide areas seemed to be the best solution. Monitoring the results of such transplants was difficult. To facilitate tracking survival, seed were often planted near a large rock or piling, or within certain ranges including a set distance from the shoreline and in water shallow enough for future monitoring. For several years, we transplanted seed by walking along the shoreline with buckets broadcasting it freely. Once we knew that even the 12 to 20 mm seed could yield adequate survival, we broadcast larger numbers from a boat.

From the bottom culture, we learned that certain areas seemed to be more conducive to optimal growth or survival. We needed to find additional areas where quahog transplants would survive and, we hoped, produce future "beds" of quahogs. We noted that shoreline with red sand and visible fresh water rivulets from the land were unsuitable for transplants. The combination of red sand, an indicator of excessive iron, and the freshets, an indicator of groundwater inputs, yielded unacceptable quahog survival. Areas of high concentrations of macrophytes in the summer (*Ulya lactuca*, *Gracilaria*, *Enteromorpha*, *Calithamnion* and *Agardhiella*) species were also poor choices for transplants, because they smothered the seed. Sediment in these areas often developed to a heavy organic anaerobic mud as part of the eutrophication process. There were other areas where an inhospitable environment was more subtle and difficult to define, but when the result was poor, we abandoned those areas.

Seed quahogs were planted in September, October, and November. When the seed were close to 25 mm (from the rafting techniques), they could be planted in September without much problem. They dug in within 2 hours and often within 30 minutes

and did not seem to be bothered by the major predators. Smaller seed, planted in September, were heavily preyed upon. The primary predators were baitfish (primarily *Euspira*), which ate the foot as the quahog was digging in, and crabs of various species.

Laboratory experiments revealed that quahogs stop siphoning at 38°F (3.5°C). We experimented with planting times and found that if the water temperature had dropped to about 45°F (7.5°C), the seed had enough mobility to dig in, but the predators were fairly inactive. We often watched quahogs after planting to see what happened or came back in a few hours to dig them up again and check for evidence of predation. We felt confident, after many trials, that this method worked well for us. Thus, holding the seed until November became routine practice.

Planting was done along stretches of shoreline. Because intertidally transplanted quahogs may not survive well in winter (bottom experiments), nearly all the seed were planted below the low-tide mark. In most of the areas planted, there was a narrow band of fringe salt marsh (*Spartina alterniflora*), and the seed were usually planted about 20 to 30 ft (7–10 m) from the marsh edge. The subtidal zone that begins 50 to 80 ft (16–24 m) from the marsh edge is either eelgrass or silty organic mud substrate and corresponds with a depth of 4 to 8 ft (1–3 m). Quahogs survive in these areas, but they grow more slowly and are inaccessible to harvest by the general public. Therefore, planting was done in the narrow band of 2 to 5 ft (1–2 m) depth, about 20–40 ft (7–12 m) from the edge of the marsh.

Site Selection

Through a process of elimination, we found numerous suitable areas for transplant (Fig. 13), some of which were used repeatedly

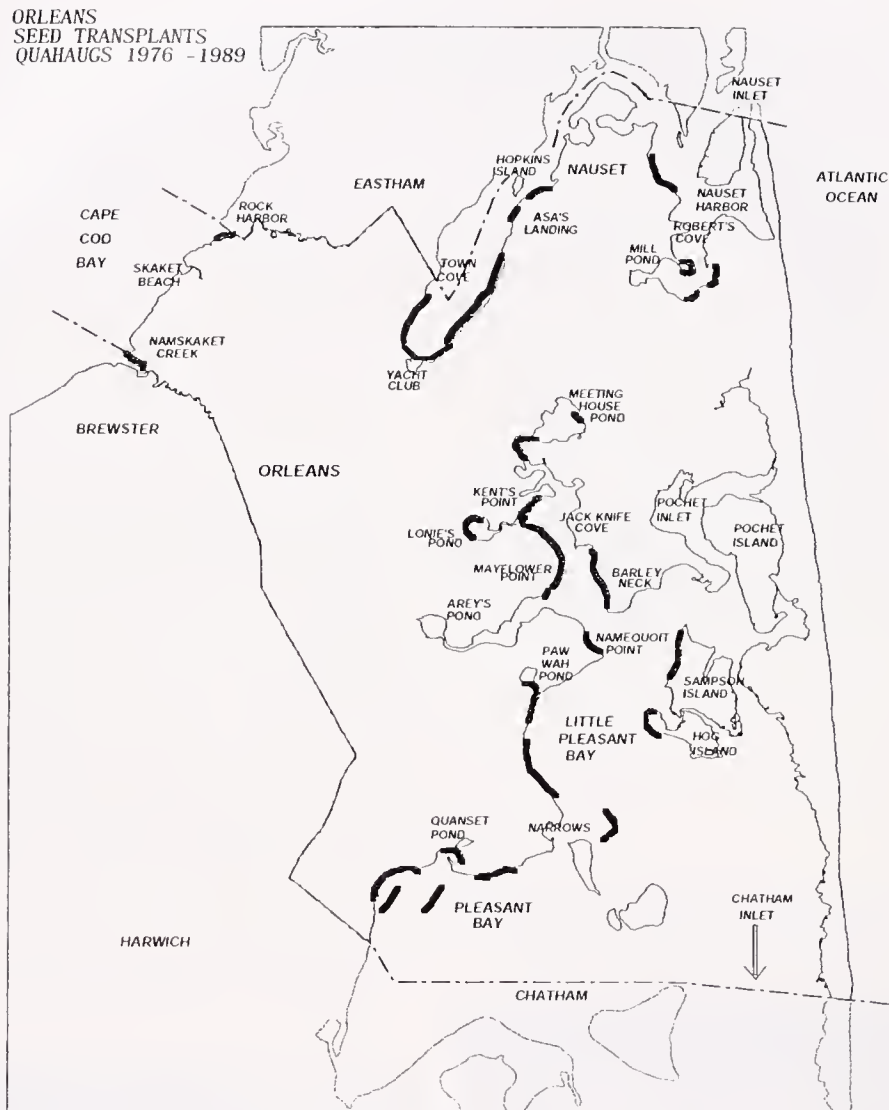


Figure 12. Seed, transplanted in all three estuaries, was the final phase of the program.

in the transplant procedure. Many of these areas had been productive, and we theorized that if they were productive at some point, gregarious setting (Hidu 1969) would restore natural production, given an infusion of seed. The Town also planted many bushels of adult spawner quahogs in the same areas to increase the chances of larvae settling in the general vicinity. Amounts planted in all three estuaries are as follows:

1. Cape Cod Bay was unsuitable for transplants primarily because ice build-up scoured the flats when the ice moved. Although the seed may have survived, it disappeared from the transplant location, and a transplant could not be justified.
2. The Nauset system received a moderate amount of seed. The estuary is naturally productive, especially in the Town Cove and Mill Pond, both of which are heavily used by harvesters. Seed planted there was intended to augment the natural production. The Nauset system has several areas reserved for family permit holders, where commercial fishing was prohibited. We felt that if those areas, Yacht Club, Asa's Landing, and Mill Pond were seeded, it would show recre-

ational users that the program was working. Also, recreational users would tend to stay in the seeded areas and would not be competing with commercial fishermen in other areas open to all harvesters. By 1981, natural stock in the Town Cove areas traditionally fished by commercial fishermen was dwindling, and we began planting seed there as well. Therefore, we planted a total of 2.1 million seed in the Nauset system.

3. Pleasant Bay, had exhibited poor natural production for many years. The majority of the seed from our program was transplanted to the bay. Upper regions of Pleasant Bay, from Meetinghouse Pond to Namequoit Point, received the most: 2.8 million. We had some success in Little Bay outside Pau Wah Pond (355,000), but the seed were preyed upon by small knobbed whelks (*Busycon carica*). The remainder were planted in other parts of Pleasant Bay including the perimeter of Big Bay.

Quahogs were harvested in shallow water using "scratchers" (short-handled rakes) and in deeper water using "bullrakes" (long-handled rakes with baskets) (See Schwind 1977, for ex-

amples of harvesting gear). The deeper waters, (12–20 ft, 4–7 m) of Big Bay, had provided employment to about 40 bullrakers for some 20 years. Monitoring quahogs planted in the deep water would have been difficult, so we limited planting in Big Bay.

The hydrodynamics of Pleasant Bay changed abruptly and drastically in January of 1987, when a breach occurred in Chatham Harbor, resulting in higher tides and greater tidal exchange. Old maps of the area indicated the new inlet location after the breach approximated that of the 1950s, when the large set occurred in the bay.

MONITORING

Monitoring was performed as a qualitative check on survival and growth, not as a quantitative measure. Survival was estimated based on repeated sampling of the transplant areas. Broadcasting seed quahogs over large expanses of shoreline to prevent predation and enhance survival precluded accurate tracking of the large number of seed. However, we observed that in areas where small plantings were done near rocks, pilings, or within known ranges, if the seed survived the initial transplant and the first winter, they were likely to survive to legal size. Our best estimate, is that 50 to 75% survived the transplants, especially if we waited until November to plant.

The author repeatedly sampled most transplant sites, using a basket scratcher with 1/4" (6 mm) hardware cloth lining designed to catch shell fragments and small seed. At each location, approximately 200 ft (60 m) of shoreline was sampled by pulling the scratcher through about 6 ft (2 m) of sediment per sample. Seed, adult quahogs, and evidence of problems were recorded. The process was repeated several times to cover the distance from the marsh edge that had been planted. When we used *M. mercenaria notata*, identification of planted seed was easy. Hatchery stock without the genetic markings was different in appearance (wider, thinner shells, less highly raised ridges), from the natural stock and could be easily identified.

Discussion

Planting seed by walking the shoreline was fairly accurate, but the optimum strip that met the criteria was more difficult to define when seed were planted by boat at high tide, and some seed were inevitably planted outside the optimum area. We felt that these would survive but not be readily harvested and might therefore, provide parent stock for repopulation of the area.

We heavily planted the Town Cove, (Nauset estuary) from Hopkins Island to the head of the cove at the Yacht Club and Mill Pond. We had hydrographic information that suggested turnover rate for the Town Cove to be 2 days (Teal 1983, Aubrey et al. 1997). We planted the area from Meetinghouse Pond to the end of Barley Neck and Lonnie's Pond. We surmised from the hydrographic data that all these areas might retain larvae, because they are semienclosed ponds with narrow openings that lead to the mouths of the estuaries. We felt that if those areas were heavily planted, and if the conditions were conducive for setting, natural productivity could be augmented. We planted the perimeter of Big Bay from Quanset to the Town Line because of the large set in the 1960s, theorizing that it is more likely now for Big Bay to be productive again, especially if there are larvae in the water to assist in establishing a new set. The change in inlet location could provide the conditions conducive for a new set.

Fishers routinely found planted stock but kept that information

to themselves. The areas of Pleasant Bay that were heavily planted are not easily accessible except by boat and are visually inaccessible to observe fishing activity. Therefore, we did not know who was fishing where until after the fact.

Virtually all littleneck (2" legal stock) quahogs harvested from Little Pleasant Bay resulted from this program. Many of them showed *M. Mercenaria notata* stock markings; however, transplant success was difficult to document, because the transplant method and the time lag between transplant and attaining legal harvesting size was long (4–7 years). Moreover, the harvest statistics did not provide a means of measurement because of:

1. lack of differentiation between cultured and wild stock; and
2. lack of cooperation from fishers to divulge whether they had harvested cultured stock, and if so, where they had found them.

The program has been criticized both by fishers and the scientific community for lack of definitive information regarding the survivability of seed transplanted to the natural environment. Field trials of small seed (10–18 mm), approximately 500 per trial, planted at various locations at varying densities (10 to 25/0.3m², without any form of protection, were planted in September, October, and November. Those planted in September were heavily preyed upon, and except for extremely low-density plantings (≤ 1 per square foot) regardless of size, approximately 90% were dead within 1 week. Those planted in October had about 75% mortality, but it took about 2 weeks for the mortalities in high density plantings. Those planted at lower densities exhibited similar mortalities, but it took about 1 month. The larger ones (15–18 mm) showed 25 to 50% mortality. Those planted in November exhibited 10 to 25% mortality within the first 2 weeks and nothing thereafter, regardless of the density or size, as long as the initial transplant was done when the water temperature had dropped to below 50°F. We observed that seed moved from the place of initial transplant, which further complicated recovery and analysis. Often, "lost" animals were later found nearby. Most designated as "lost" were predated, because shell fragments were often found in the planting locations. Had we used enclosures to track mortality in a more statistically accurate manner, we felt we would have been creating an artificial situation that would not represent what would happen once the animals were freely broadcast.

The trial plantings suggested that time of planting was as important as density and size at planting. A high density in a relatively small area seemed to draw predators to the area, but broadcasting seed in a wider area at very low densities (often ≤ 5 per square ft) increased survivability. Further increases in survival were achieved by planting all seed when the water temperature was between 45 to 50°F (7.5–10°C). Based on the trials, the transplants from the upwellers, where the seed was between 12 to 20 mm, were done in November, and transplants of the larger seed from the rafts were done in October.

A total of 6,297,000 seed were broadcast in Orleans, which is 10,495 bushels of quahogs at a legal size (2" at 600/bushel), if all survived. We estimate we added between 5,200 to 7,900 bushels of quahogs to the fishery at 50 to 75% survival, with an estimated wholesale value of \$800,000 to \$1,170,000 (average price from 1980–1990). The seed cost the town \$135,000 (exclusive of biologist's salary and amortization of equipment, Table 8).

From 1994 to 1996, we estimate 40 to 60% of the quahogs harvested from the Nauset system resulted from seed planted in the late 1980s. Those harvested from the Yacht Club, the east shore of Town Cove, and Nauset Harbor were especially noticeable. In the

winters of 1992 and 1993, bullrakers who had moved to Orleans from Long Island, NY, were fishing in Town Cove. Local fishermen joined them and worked in the Town Cove for three successive winters. Up to 20 boats worked each day. Both the fishermen and Shellfish Department recognized the notata quahogs and knew that they were not native stock. Slowly and individually, the local fishermen began to acknowledge the success of the propagation program.

CHANGES IN THE PROPAGATION PROGRAM

In most wild fisheries, the commercial vision and focus is on short-term gains rather than long-term benefits. This was a key point of Hardin (1968). This was true for our program, where it was perceived as a failure by the local fishermen. They had an overwhelming desire to see immediate tangible results, such as the 1" seed from the rafts, but it took 4 to 7 years for the seed to attain legal size in many locations. This was too long to wait for visible results.

In 1989, the state withdrew financial assistance to municipalities for propagation. This had funded a major portion of the program in Orleans. Aside from the biologist's salary, the major program expense was seed cost and the seasonal technician's salary. Operating the lab by volunteers was suggested, but was not accepted because of perceived liability problems.

The town had purchased two open space parcels on Pleasant Bay that would be suitable as shellfish propagation facilities. After the oil spill settlement, plans were drawn to construct a new facility at Kent's Point that would include a public education component. The land was purchased for "conservation, open space, and passive recreation" with a caveat in the deed that a shellfish facility could be the only additional building built on the property. A benefactor offered to construct the building and create an endowment. A second benefactor was ready to lend support. The Selectmen would not entertain either offer, stating that, at some point, the town would have to pay something. A management plan for the property included a shellfish grow-out facility, but the idea was not pursued because of fears that the remote area, accessed by a private residential road, would be "overburdened" if the shellfish facility were there. The Conservation Commission, who administer and oversee the property use, opposed a shellfish facility. They added it to the management plan only because the deed allows such activity, and it was voted favorably by the citizens at a town meeting.

The decrease in funding, the oil spill of 1985, a smaller incident in 1987 that did not cause mortalities but indicated the undependable nature of the site, the change in Shellfish Department Manager to one who had no experience with shellfish propagation, the fishers' perception that the program was a failure, the success of private shellfish farmers in Wellfleet (a neighboring town) using bottom culture (see below), and a disturbing trend of degraded water quality (Macfarlane 1996), all converged in 1989. The position of shellfish biologist was changed to shellfish biologist/conservation administrator. The duties changed from shellfish propagation and management to administering the state and local Wetlands Protection regulations and environmental planning. No further responsibility for work with shellfish propagation was included. The laboratory was closed and razed in 1993.

Shellfish Propagation after 1990

The municipal propagation program (1990–1994) consisted of floating trays deployed at Asa's Landing (Nauset) and subtidal

bottom boxes at six other areas. Trays received 10 to 20,000 (2.5 mm) quahogs per tray at \$10.00/1,000, and bottom boxes were stocked with 10 to 15,000 quahogs (2.5 mm) per box.

The floating trays were used, because they were easier to handle than the old rafts, and another town had indicated successful results with the floating trays. They were 3' × 4', constructed of wood and netting with solid covers to prevent bird damage. The covers reduce water flow, but the seed are not predated and do not wash out. Seed ordered for May delivery was usually received in late June or July. The seed were placed in the floating trays the first summer. They transfer the 10 to 12 mm seed to subtidal bottom boxes in late summer or early fall. They are retained in the bottom boxes for a second season and winter until they are about 18 mm, when they are finally transplanted to the natural environment. Those transplants take place in the spring.

The bottom boxes were constructed of 2" × 6" stock, 5' × 10' with 1" galvanized coated wire bottom mesh covered with landscape cloth. They are filled with sand, planted with seed, and covered with 1/4" plastic mesh covers constructed with laths (1" × 2") attached to the sides.

The stock bought in 1994 remained in bottom boxes for 3 years before transplant. Covers remained on the boxes during the winter, and there was little damage, because there was little ice cover in those years. In 1997, the Shellfish Department deployed 10 floating trays in Lonnie's Pond and 25 bottom boxes. Approximately 50% of the 1991 stock was lost in a hurricane. There is no record of whether the loss was actual mortality or displacement of the seed.

In 1996 to 1997, the Department purchased "field planting"-size quahogs, about 12 to 20 mm at \$25.00/1,000. They changed their strategy for nursery culture and now buy small seed 1 year and large field plant seed the next to stagger the nursery requirements. Lack of funding for personnel to care for smaller seed has resulted in losses caused by heavy fouling, predation if holes develop in protective netting, and other problems. From 1990 to 1997, 5.4 million quahogs were grown in nursery culture. They estimate survival from purchase to transplant in the field to be about 50 to 60% or 2.7 million seed. There are no estimates of survival once the seed are transplanted. Larger seed can be planted without the nursery phase and with a propagation budget of \$9,000, but options are limited. Bay scallop (*Argopecten irradians irradians*) seed (865,000 since 1992) are also purchased with the funds.

Commercial fishers have recently assisted the Shellfish Department in harvesting and transplanting seed, making it a community project rather than a departmental one.

Orleans constructed a tidal upweller in 1990 based on plans by Mook (1988). Although a smaller version worked well in the neighboring town of Eastham, it did not work well in Orleans in the initial year, and the idea was never modified. It is difficult to find an area with enough tidal current necessary to operate the upweller successfully in Orleans that does not have substantial boating activity. Without that method, Orleans must rely on bottom or off-bottom culture or on a shore-based nursery for propagation methods.

PRIVATE AQUACULTURE IN ORLEANS

The first private aquaculture authority was specifically granted to Eastham, Orleans, and Wellfleet (1904) to allow "bedding" of quahogs until the market was more favorable (Belding 1912). In

1909, the legislature granted licensing authority for planting and cultivating quahogs to all the towns in Massachusetts. For many years, the only propagation in most towns was public and consisted of transplanting adult "spawning stock" to stimulate a set of quahogs, as similarly described by Kassner and Malouf (1982).

In the late 1980s, private aquaculture developed on the Cape. The success of municipal efforts described for Orleans prompted private shellfish farmers in Wellfleet to adopt low-cost intertidal bottom culture. Their success increased requests for leases or shellfish grants in many towns. Modified predation enclosures were improved (iron rebar rather than wood) for predator exclusion.

In Orleans, the town adopted a shellfish management plan (Macfarlane 1986) that outlined a system for private aquaculture to be initiated in Orleans. The plan included several recommendations.

1. Three areas to be considered for grants:
 - A. From Old Field Point to Pochet Inlet (Pleasant Bay);
 - B. Pleasant Bay west of Nauset barrier beach;
 - C. Cape Cod Bay flats;
2. Grants to be 1/2 acre for the first year, with an option for renewal and expansion;
3. upper limit of 5 acres/grant;
4. upper limit of 5 grants/area, with buffer zones between grants;
5. grants to be self-policing;
6. all grants to be surveyed by a registered land surveyor and survey to be recorded and on file at the Town Hall; and
7. subleasing of grants to be prohibited.

After 2 years of debate, the plan was implemented. The issue of designating public bottom for private enterprise was the primary focus of the debate. It was a common public perception that grants in existence in the 1960 to 1970s had been mismanaged. The pervasive feeling voiced strongly at public meetings was that public waters should not be leased to private entrepreneurs, except for small isolated cases. At the time, Orleans had eight grants ranging in size from 0.06 acre to 1.0 acre (most were 0.5 acre). A single 10-acre grant lease had not been renewed because of nonuse. Lease applications meeting state criteria were usually approved by the town, because there was no plan to determine where additional grants could or should be sited.

The Shellfish Department determined that two areas in Pleasant Bay could be set aside for shellfish grants. The primary area (from Old Field Point to Pochet Inlet north of Sampson Island) has a maximum depth of 2 ft (0.6 m) at low tide, was not under riparian rights of upland property owners, was not heavily used for boating, was "out of the way" for most bay users, and importantly for Massachusetts laws, had been unproductive of shellfish resources for at least the last 40 years.

Shortly after adopting the Management Plan, the Selectmen changed the regulations to two acres per grant (maximum), and the total grant area in the primary zone was expanded to 30 acres. By 1990, there were three new grants, all in the primary area in Pleasant Bay; two grantholders relinquished their acreage by 1993. New grants were not surveyed, because grantholders argued that the requirement was prohibitively expensive. Buffer zones were established haphazardly, if at all. As a result of this activity, the subject of shellfish grants has remained contentious over the past decade.

A portion of the grant debate has centered on determining grant use. By 1994, there were 15 new grants. The Shellfish Advisory Committee tried to ascertain how people were using the grants.

The following year, six new individuals requested 1/2 acre grants. The Shellfish Department recommended against the proposed leases, because if the existing 15 grantholders were allowed to expand to the legal 2 acre maximum, there would not be enough room to accommodate 21 grants. The Selectmen were pressured into allowing the additional licenses and amended the grant regulations. The primary reason for the increased activity and political pressure was the availability of Federal funds to aid displaced fishers in the Northeast. The six were granted licenses, but only one received Federal funding.

With a waiting list for both grant expansions and for new grants, it was determined that current license holders must prove that they are actively engaged in aquaculture either by seed receipts or by compliance with a "density requirement" enacted to ensure that the grant is being "worked." Those who opposed adding new grants suggested that if the grants were not "worked," the bottom should be relinquished. After considerable debate, regulations were adopted by the Selectmen to include a density requirement (minimum 50 quahogs/square foot). To maintain an existing grant for the 5-year lease, 75% of the area had to be planted with the minimum density. Furthermore, no expansion would be considered unless 75% of the existing grant area were planted with the minimum density. Compliance was required by December 31, 1996.

Before the deadline, aquaculturists requested a review of the regulations and proposed an alternative. New regulations were enacted in May, 1997, including a 3-year planting schedule, by which 50% of the grant must have a density of 30 quahogs per square foot or an expenditure of \$2,500 per 1/2 acre expended annually (on shellfish only), with proof given through seed receipts. A hardship clause was also included. If grants were relinquished, expansion preference for the area would be given to original grantholders. Only after expansion would a potential aquaculturist on the waiting list be given an opportunity. Sale and/or transfer of licenses was prohibited.

As of 1997, there were 26 grants in Orleans. Those who received 1/2 acre from changes in regulations, insist that the town find suitable acreage for expansion. To date, the town is waiting for a regional resource management plan to be completed and adopted. The proposed "density requirement" has sparked continual controversy and has yet to be resolved.

Resource-Based Management Plans

In 1995, Orleans entered into an agreement with the neighboring towns of Brewster, Harwich, and Chatham to develop a Resource Management Plan for Pleasant Bay, a state-designated Area of Critical Environmental Concern (ACEC). The plan was divided into five issues: structures (docks, piers, and erosion control structures), shellfish/aquaculture, biodiversity, boating and navigation, and public access. Five working groups met during the winter of 1997 to discuss key issues, and each group provided recommendations and/or comments. No consensus was reached on aquaculture.

Orleans is the only town of the four that allows private aquaculture. Working groups independently suggested formation of a scientific panel/oversight group that would initiate requests for specific studies and review documentation as it became available. One key data gap is an ecological assessment of the bay; the latest information is from the 1960s (Fisk et al. 1968), (U.S. Army Corps of Engineers 1968).

Not all the dynamic fisheries in the Bay are regulated. For the past 2 years (1995 to 1997), razor clams (*Ensis directus*) have been harvested in quantity, and one fisher has harvested horseshoe crabs (*Limulus polyphemus*) for about 20 years. The crabs are trucked to Falmouth, bled for their medically important lysate, and returned to the Bay. The fisherman has harvested crabs throughout the shallow bay waters (Harrington, pers. comm.). These are the same areas requested for grant expansion in both the primary and secondary areas allowed for grants in Pleasant Bay. Neither of the above fisheries is regulated, but state law, significantly modified in 1995, requires that a grant cannot be issued for an area that would affect the town's natural resources. Before the newest changes, grants could not affect a town's shellfish resources, narrowly defined as clams (*Mya arenaria* Linne), quahogs (*Mercenaria mercenaria*), scallops (*Argopecten irradians irradians*), mussels (*Mytilus edulis*), and oysters (*Crasostrea virginica* Gmelin). In addition, horseshoe crabs have been listed as shellfish predators. Although this would seem to preclude all leasing, the regulations have not had that effect, but there has been controversy over what would constitute significant adverse effect to the town's natural resources.

Quahog Diseases

In 1995, a phenomenon was described in Duxbury and Provincetown, MA where cultured quahog stock was dying just before reaching market size. In their paper Smolowitz et al. (1997) described the parasitic infestation QPX (Quahog Parasite Unknown) as the primary cause for the mortalities.

Smolowitz et al. (1996) described the history, symptoms, and possible protective measures in an advisory bulletin. The organism was first identified in a dense natural set of wild hard clams in New Brunswick, Canada in 1959, and in 1989, at a nursery on Prince Edward Island, NB. Unpublished observations of Ford et al. (1976) and Smolowitz (1992) found QPX-like organisms associated with dead and dying hard clams in natural populations from Barnegat Bay, NJ and Mitchell River, Chatham, MA. The Provincetown infestation occurred to hard clams being cultured and the QPX-like organism was the only microorganism found in the tissues.

The life history of the QPX organism is unknown. The organism may be present in the water column or sediment of growing areas. It is unlikely that the infestation originates from hatcheries (Ford et al. 1997). Crowding can be a form of stress that may compromise animals, and optimum planting density for each area is unknown.

The ramifications of this disease are germane to the shellfish propagation efforts of both the town and the private culturists. At this point in the investigation, density may play a role, but its effects are unknown.

If density proves to be a factor, this poses somewhat of a dilemma for Orleans. The town wants proof that leased bottom is worked and have required a "density" regulation. But high density may encourage proliferation of the QPX. Grants squeezed into a relatively small area and located on "barren" ground as defined by the state may add stress to the animals and may increase the probability of infection. With 75% of the grant area required to be worked, crop rotation is limited.

BUDGET/FINANCIAL CONSTRAINTS

In 1974, the state provided financial assistance for shellfish propagation and established a reimbursement program. A percent-

age of a town's shellfish department operational budget was reimbursed for the shellfish projects.

Funding was limited and a continual hindrance throughout. The increased lab operations costs from 1985 to 1989 were attributable to continuing the technician's salary through October. An operating budget of \$12,000 produced wild fishery quahogs worth \$150,000 wholesale.

The total propagation budget for 1997 was \$9,000. With higher seed costs (\$10.00/1,000 for 2.4 mm quahogs), including scallops, the town has a limited propagation effort. The Shellfish/Aquaculture workgroup for the Pleasant Bay plan placed a high priority on increased funding for shellfish propagation. This prioritization was based on shellfish catch statistics since 1970 (Table 7). Although propagation costs have been modest, the value of shellfish to the town is considerable.

The funding was the rationale for Orleans to hire a shellfish biologist and start the seed program. The funding decreased over time but lasted until 1989 (Table 8). It ended because of fiscal constraints at the state level and perceived misuse of funds by many towns from the intended purpose.

Compilation of these data was stimulated because of the need to assist Orleans Shellfish Department and others seeking to justify funding shellfish propagation. The economic returns to the town are significant, but tangible benefits also accrue because of the need to maintain water quality in shellfish areas. In addition, local propagation efforts can serve as a catalyst to reconnect town residents with their harvesting of natural resources nearby.

CONCLUSIONS

Orleans demonstrated that municipal propagation of hatchery-reared nursery cultured quahogs (*Mercenaria mercenaria*) is a feasible and economically viable way to augment natural production. Orleans was the first town in Massachusetts to specifically hire a biologist for shellfish propagation and management. Over a 15-year period, from 1975 to 1990, we developed and utilized techniques that including bottom, rafts, a hatchery, and upweller culture with a maximum annual budget of slightly over \$12,000 (exclusive of the biologist's salary). The town successfully transplanted over 6.3 million seed from 1975 to 1989.

All methods showed promise, but the upweller technique proved to be the most successful in terms of survival and the number of animals that could be cultured, given the program constraints. All methods are labor intensive, demand close attention, and all are dependent on natural conditions. The latter are erratic, and disaster can strike with little or no notice.

Our original intent was to discover if hatchery-raised quahogs could be utilized for propagation efforts. We showed that regardless of seed supply, hatchery-raised seed survived and grew.

The discovery that temperature plays a critical role in survivability of transplanted stock was crucial to our success. The observations, in the lab and in the field, on activities of the seed and other biota resulted in determining 45°F (7°C) as the optimal temperature for transplant. Transplants of different seed sizes led us to conclude that we could successfully transplant seed as small as 12 to 15 mm, with no additional protection, as long as we waited until November to plant.

We experimented with three different types of bottom nursery enclosure designs at 20 separate locations over a 3-year period. Enclosure design is critical to the success of bottom culture. Some areas were clearly better than others, but reasons for success were poorly understood.

TABLE 7.

Total harvest (commercial and recreational) of four shellfish species: clams = soft-shelled clams (*Mya arenaria*); quahogs = hard-shell clams (*Mercenaria mercenaria*); scallops = bay scallops (*Argopecten irradians irradians*), and mussels = blue mussels (*Mytilus edulis*); value = wholesale value at the time of harvest.

Shellfish Harvested in Bushels															
Year	Clams			Quahogs			Scallops			Mussels			Value		Total Value
	Com	Rec	Total	Com	Rec	Total	Com	Rec	Total	Com	Rec	Total	Com	Rec	
1970	730	930	1,660	17,085	140	17,225	1,255	485	1,740	6,100	125	6,225	\$195,069	\$15,861	\$210,930
1971	464	1,134	1,598	14,186	250	14,436	1,540	286	1,826	9,150	125	9,275	\$308,937	\$7,822	\$316,759
1972	637	524	1,161	5,840	151	5,991	3,567	700	4,267	0	35	35	\$167,328	\$23,102	\$190,430
1973	0	195	195	17,395	95	17,490	425	5	430	0	0	0	\$219,525	\$5,025	\$224,550
1974	5,699	2,170	7,869	12,460	1,145	13,605	2,050	210	2,260	1,200	10	1,210	\$263,910	\$67,815	\$331,725
1975	1,760	360	2,120	12,577	855	13,432	915	110	1,025	1,800	50	1,850	\$364,615	\$70,505	\$435,120
1976	2,603	893	3,496	10,945	716	11,661	31,490	757	32,247	5,030	450	5,480	\$760,521	\$75,536	\$836,057
1977	3,098	1,017	4,115	9,912	1,007	10,919	8,372	122	8,494	12,632	163	12,795	\$570,887	\$58,518	\$629,405
1978	2,404	981	3,385	5,633	764	6,397	160	38	198	3,090	231	3,321	\$324,094	\$57,826	\$381,920
1979	6,118	1,344	7,462	5,014	1,339	6,353	240	60	300	2,440	406	2,846	\$508,196	\$89,958	\$598,154
1980	6,499	1,336	7,835	5,612	971	6,583	15,230	612	15,842	6,150	550	6,700	\$1,243,005	\$121,256	\$1,364,261
1981	5,285	718	6,003	3,665	738	4,403	3,455	65	3,520	10,100	800	10,900	\$600,912	\$71,058	\$671,970
1982	2,781	522	3,303	3,264	727	3,991	4,958	760	5,718	19,400	540	19,940	\$606,748	\$87,424	\$694,172
1983	3,684	206	3,890	3,746	592	4,338	40,000	4,000	44,000	10,200	340	10,540	\$1,908,264	\$181,984	\$2,090,248
1984	2,599	193	2,792	2,458	342	2,800	821	70	891	1,145	104	1,249	\$278,402	\$24,246	\$302,648
1985	1,006	111	1,117	2,924	489	3,413	6,000	3,545	9,545	8,105	115	8,220	\$131,761	\$487,150	\$618,911
1986	648	79	727	2,181	280	2,461	235	31	266	2,390	215	2,605	\$176,896	\$20,597	\$197,493
1987	880	88	968	2,023	428	2,451	0	0	0	605	103	708	\$173,181	\$25,033	\$198,214
1988	615	50	665	2,310	382	2,692	2,530	101	2,631	500	100	600	\$291,980	\$26,043	\$318,023
1989	545	50	595	3,800	350	4,150	990	50	1,040	3,510	20	3,530	\$326,170	\$20,310	\$346,480
1990	803	75	878	3,889	375	4,264	860	50	910	3,261	20	3,281	\$463,073	\$26,610	\$489,683
1991	1,826	75	1,901	5,796	400	6,196	1,888	475	2,363	4,740	50	4,790	\$507,320	\$51,500	\$558,820
1992	2,325	100	2,425	3,258	250	3,508	2,450	250	2,700	4,375	50	4,425	\$597,430	\$34,150	\$631,580
1993	1,187	100	1,287	3,006	300	3,306	355	25	380	2,866	25	2,891	\$346,788	\$27,550	\$374,338
1994	1,867	175	2,042	3,588	450	4,038	701	5	706	1,635	15	1,650	\$436,206	\$41,550	\$477,756
1995	9,233	500	9,733	4,072	450	4,522	298	4	302	1,659	10	1,669	\$1,038,210	\$67,404	\$1,105,614
1996	5,080	1,550	6,630	13,567	625	14,192	1,286	110	1,396	1,112	10	1,122	\$1,441,932	\$171,140	\$1,613,072

Where mortality was attributable to predation, numerous predators were found, including crabs (many species), drills (*Urosalpinx cinerea* and), moon snails (*Lunatia heros*), and several species of fish, including *Euspira*.

Floating sand box rafts worked very well, but the deployment and hauling were unwieldy. Growth and survival were far superior to the bottom boxes, and the results were reproducible and dependable year after year without change in design or implementation. Our problems with the rafts centered on the necessity to plant smaller animals each year (because of rising costs of seed), and our reluctance to field plant anything less than 20 mm in the fall (before we had discovered temperature as the crucial factor) for fear of poor survival. Using the rafts earlier in the year, when the weather had not stabilized, increased losses. Additional raft problems came from decreased flushing and increased nutrient enrichment in the deployment area. Although growth was still good, fouling of the rafts increased maintenance. In addition, to increase seed output to a million per year or more could not be met with the rafts, because they were too big, and we were unwilling to add additional rafts to the pond.

Our efforts to operate a hatchery were not highly successful. The refusal to install a dual-intake flowing water system and the purchase of pumps that were too small were critical mistakes. The decision to construct a bulkhead and parking area creating a desirable public access point created havoc in our system. Mass

spawning may have been preferable. We felt we would have more control over individual spawnings, but this was difficult to achieve with our system. The real obstacle to hatchery success was culturing the postset animals.

Upweller methodology allowed us to use our flow-through system, and it allowed us to raise a million seed per year, at 95% survival, within the building. Expansion within a secured enclosure outside could have doubled our output, but lack of additional personnel and budget constraints prevented this. Volunteer labor, (presently the backbone of many community efforts) was not allowed for fear of liability. It is the author's opinion, judging by the number and frequency of comments heard, that volunteers would have been willing to work. The location of the facility fueled the perception that the entire project was foolhardy. The loss of a year's work in 1985 because of an oil spill pointed to the fragility of our site. Measures were taken to reduce the threat of human interference, but the area was still highly used and highly visible. We had prepared plans to move the facility to a more hospitable site, but that option was not supported.

A major problem in raising quahogs in our locale is that they take at least 4 years to mature. Politically, 4 years is longer than policy makers can wait to see tangible results. Every year added to our knowledge of how to increase survival in our area, but anything that went wrong meant a "wasted" year. Damaging winters, oil spills, poor summer weather, human error, and human inter-

TABLE 8.

Orleans quahog propagation budget including costs of program and income from permit fees.

Cost					Income				
					Permits				Total Income
					Com		Rec		
Year	Seed	Lab (Includes Labor)	Rafts/ Boxes	Total	No.	Fees	No.	Fees	
1975	\$80		\$100	\$180	91	\$910	979	\$4,291	\$5,201
1976	\$3,637		\$1,250	\$4,887	140	\$1,400	924	\$3,084	\$4,484
1977	\$6,994		\$1,250	\$8,244	114	\$1,140	979	\$4,178	\$5,318
1978	\$1,040		\$250	\$1,290	107	\$1,070	1121	\$4,684	\$5,754
1979	\$4,500	\$7,060	\$1,000	\$12,560	142	\$1,420	1,285	\$6,180	\$7,600
1980	\$3,500	\$8,912	\$0	\$12,412	248	\$2,480	1,533	\$8,068	\$10,548
1981	\$4,500	\$8,000	\$0	\$12,500	167	\$6,782	1,581	\$9,658	\$16,440
1982	\$2,100	\$6,400	\$0	\$8,500	180	\$9,000	1,928	\$8,475	\$17,475
1983	\$3,500	\$5,000	\$0	\$8,500	336	\$16,800	1,428	\$12,725	\$29,525
1984	\$2,000	\$5,000	\$0	\$7,000	354	\$17,700	1,409	\$8,355	\$26,055
1985	\$6,000	\$5,500	\$0	\$11,500	225	\$12,200	1,253	\$9,223	\$21,423
1986	\$6,200	\$5,700	\$0	\$11,900	167	\$11,250	1,192	\$7,194	\$18,444
1987	\$6,200	\$5,700		\$11,900	167	\$8,350	1,059	\$6,900	\$15,250
1988	\$6,200	\$5,900		\$12,100	285	\$8,350	1,113	\$6,380	\$14,730
1989	\$6,200	\$6,000		\$12,200	195	\$14,250	1,248	\$6,155	\$20,405
Total	\$62,651	\$69,172	\$3,850	\$135,673		\$113,102		\$105,550	\$218,652

vention all took their tolls on the product. These factors made budgetary justification difficult; neither finance committee members or Selectmen were willing to take positive results on faith. There was also a feeling that the town should not be conducting research, because leaders felt that the state should be doing the work. Because shellfish management is a town responsibility, the Shellfish Department felt justified and mandated by law to manage shellfish to the best of our ability, including experimentation.

Several decisions were made that may have contributed to the program's decline. One was to plant many "family permits only" areas in certain high visibility locations. Although the program gained support and admiration from many recreational shellfish permit holders, it did not gain credibility with the commercial fishers, who were not allowed to fish in those areas.

The second decision was to plant areas that were sparsely populated with quahogs but not to advertise that they had been planted in those areas. General planting locations were given to anyone who asked, but we were not specific. Because the seed was broadcast at low densities along long stretches of shoreline, and it took 4 to 7 years to reach harvestability, a lack of confidence developed among the commercial fishers that anything was being done.

The third was to plant all the seed without enlisting the services of any other user groups. Without that interaction, there was only criticism and lack of support from all directions.

Although we stand by our original intent to try to augment the natural production of Pleasant Bay, which was sparsely populated with quahogs, the amount of seed produced for such an extensive area proved to be inadequate to make a difference visually or statistically. We believed that when the transplanted seed matured, it would reproduce, and when of legal size, it would be harvested. We had hoped that enough quahogs would be harvested from our program to prove its worth to the town. However, our intent was not a "put and take" program, where every quahog that was planted was harvested several years later. Parts of the bay now producing quahogs were not producing any when we started the program. A total of about 9.0 million seed added to the waters

from 1975 to 1995 were more tangible than relying on spawner transplants.

The Shellfish Department was, is, and will be short staffed. With the change in position of the biologist to conservation administrator, the Shellfish Department lost an employee whose responsibility was strictly shellfish propagation and management. The Shellfish Constable is (and was) also the Harbormaster and, therefore, has a dual role in a town with three separate estuaries, a migrating barrier beach with one of the three most dangerous inlets on the East Coast, and a harbor that boasts being the largest charter fishing fleet in New England. The Department Manager from 1974 to 1983 called himself Shellfish Constable first and Harbormaster second. The next three succeeding Department Managers switched the order of their title. Although possibly insignificant, it subtly shows which duty is first priority.

Shellfish records kept after 1990 were inconsistent, and propagation program records were very difficult to obtain. The department duties overwhelm the staff, and propagation program record keeping is not high on the priority list. No one person has responsibility for records or for maintaining nursery culture systems, and maintenance is "as needed" or when time permits. Research for this document has heightened the necessity of good records for accountability and historical perspective. Records also project a positive professional perspective to the public when requesting funding.

We remain optimistic about the future of shellfish in Orleans. Our experiments of the mid-1970s using hatchery-raised seed have become routine grow-out procedures for both municipal programs and private aquaculture. Both Chatham and Harwich, towns that border Orleans on Pleasant Bay, have municipal programs. Eastham has continued its efforts. Orleans has continued to use nursery grow-out techniques to supply seed for the public fishery.

This paper documents work involved in nursery culture of quahogs and the management options used by the town. Economics, politics, and social pressures played enormous roles in the operation and evolution of the program. Shellfish were, and still are, an

important facet of the town character, and although interest in shellfish seems to be waning with the gentrification of the town, surveys continue to show that whether people actually go shellfishing, the fact that shellfish are there to take as a continuing natural resource is important, and citizens are willing to pay for the privilege.

In retrospect, Belding (1912) was right: "growth in any particular locality can be determined only by experiment" and without culturing, the fishery will collapse. What Belding could not have foreseen was the enormous social change and attendant economic and political forces that become part of the "experiment."

Dedication

This manuscript is dedicated to Gardner Munsey, Orleans Shellfish Constable from 1974 until his retirement in 1983. Without his vision for Orleans as the shellfish Mecca of Massachusetts, his inquisitiveness, and his never-ending support for all aspects of a program to attain that goal, none of the preceding would have been possible.

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REPRODUCTIVE CYCLE OF *SPONDYLUS LEUCACANTHUS* BRODERIP, 1833 (BIVALVIA: SPONDYLIDAE) AT ISLA DANZANTE, GULF OF CALIFORNIA

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ABSTRACT The reproductive cycle of *Spondylus leucacanthus* at Isla Danzante, Gulf of California, was studied from January 1994 to January 1996. Microscopic analysis established that the female specimens had male gametes in the gonads during the starting of gametogenesis (April 1994, March 1995). The individuals were fully ripe from June, coinciding with the highest values of gonad index. In both the years investigated, spawning occurred during August to October (23 to 26°C), when the gonad index was falling. Spawning occurred at the mean shell height of 75 mm.

KEY WORDS: Reproductive cycle, bivalves, *Spondylus leucacanthus*, histology, Gulf of California

INTRODUCTION

Spondylus leucacanthus Broderip, 1833 is commonly named "Viejita" or "Concha China." It is distributed from the Gulf of California to Ecuador and can reach a length of 150 mm (Keen 1971). Its main habitat is the bottom at about 40-m depth, although organisms can be gathered from shallow water up to 3-m depth. As with the majority of the bivalves, this is a filter-feeding species, feeding mainly on detritus and phytoplankton (Villalejo-Fuerte and Muñetón-Gómez 1995).

Anatomical and morphological descriptions of *Spondylus* spp. have been published by Yonge (1973), Dakin (1928a) and Dakin (1928b). A small number of studies on other topics have been made by Mata et al. (1990), Parth (1990), and Okutani (1991). A recent review of the taxonomy, habitat, and distribution of Genus *Spondylus* of the Panamic Province was made by Skoglund and Mulliner (1996).

Despite the wide distribution of this species around the islands of the Gulf of California, there are no published records on its biology. This study describes the reproductive cycle and the spawning season of *S. leucacanthus* from subjective analysis of histologic gonad sections, measurements of oocyte sizes, and calculated gonad index from weight measurements.

MATERIALS AND METHODS

Monthly, from January 1994 to January 1996 at Isla Danzante, Gulf of California (25°48'54", 111°15'45") (Fig. 1), 30 to 60 specimens of *Spondylus leucacanthus* were collected by trawling with a net at 40-m depth. The bottom water temperature was recorded at the time of sampling using a Van Dorn bottle and a protected thermometer with range of -10 to 110°C. Shell heights and the wet weights of the gonad and total soft body were recorded for each clam after fixation in a neutral 10% formalin solution prepared with seawater.

The sex ratio of the population in the study period was obtained from histological analysis of all the clams collected. The females and the males were separated, and the percentage of each sex in the study period was determined. The significance was tested with a chi-square analysis. The null hypothesis of 1:1 sex ratio was established, and the observed value was compared with the theoretical value of $\chi^2 = 3.84$, $\alpha = 0.05$ (Sokal and Rolf 1979).

The gonad index was calculated using the criteria of Sastry

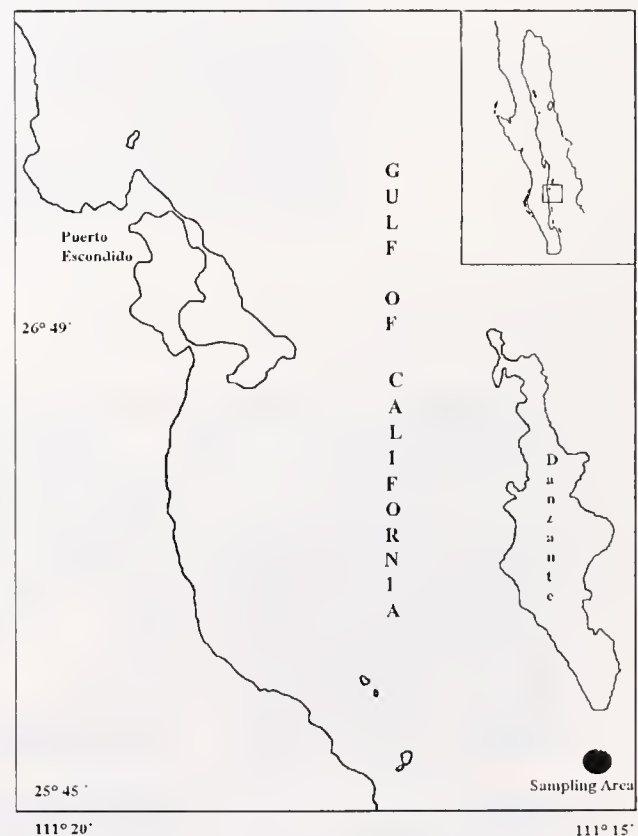


Figure 1. Location of the sampling station near Isla Danzante, Gulf of California.

(1970), which uses the weight of gonad and soft body, and is expressed as percentage. For histological studies, a piece of the gonad (one cubic centimeter) was dehydrated in alcohol and embedded in paraffin. Sections (7 μ m) were placed on slides and stained with hematoxylin-eosin (Humason 1979).

The gonad developmental stages of the individuals sampled were defined from the histological preparations using categories comparable to those species that have previously been studied in the Gulf of California, such as *Megapitaria aurantiaca* (García-Domínguez et al. 1994), *Argopecten circularis* (Villalejo-Fuerte

and Ochoa-Báez 1993), *Glycymeris gigantea* (Villalejo-Fuerte et al. 1995), and *Laevicardium elatum* (Villalejo-Fuerte et al. 1996a). The classification of gonad condition consisted of five main stages: indifferent, developing, ripe, spawning, and spent. The stages are described below.

Indifferent Stage

This stage is characterized by a total absence of gametes, therefore, it is not possible to distinguish between the sexes. Empty follicles are seen, and the connective tissue occupies almost all the space (Fig. 2A).

Developing Stage

Female

The germinal cells can be observed, and developing oocytes are attached to the follicle walls (Fig. 2B).

Male

Varying quantities of spermatogenic cells and spermatocytes were present (Fig. 3A). Interfollicular connective tissue begins decreasing.

Ripe Stage

Female

All follicles are filled with ripe oocytes of polygonal shape. Some oocytes remained attached to the follicle walls (Fig. 2C).

Male

Follicles filled with spermatocytes, spermatid, and spermatozoa are arranged in characteristic bands (Fig. 3B). Interfollicular connective tissue is absent.

Spawning Stage

Female

The centers of follicles are partially empty, and there are large spaces between the free oocytes that were present. Developing oocytes are present at the follicle walls (Fig. 2D).

Male

Follicles are partially empty, with large spaces inside the follicles and a marked decrease in the quantity of spermatozoa. The spermatocytes remain at the walls of the follicles (Fig. 3C). Interfollicular connective tissue is scarce.

Spent Stage

Female

At this stage, the follicles are empty, but some unspawned oocytes were observed within them (Fig. 2E).

Male

In some follicles, only a few residual spermatozoa were present (Fig. 3D). In both sexes, the gametes were being phagocytosed by amebocytes. There is abundant interfollicular connective tissue.

To analyze the sex of this species, females with spermatozoa

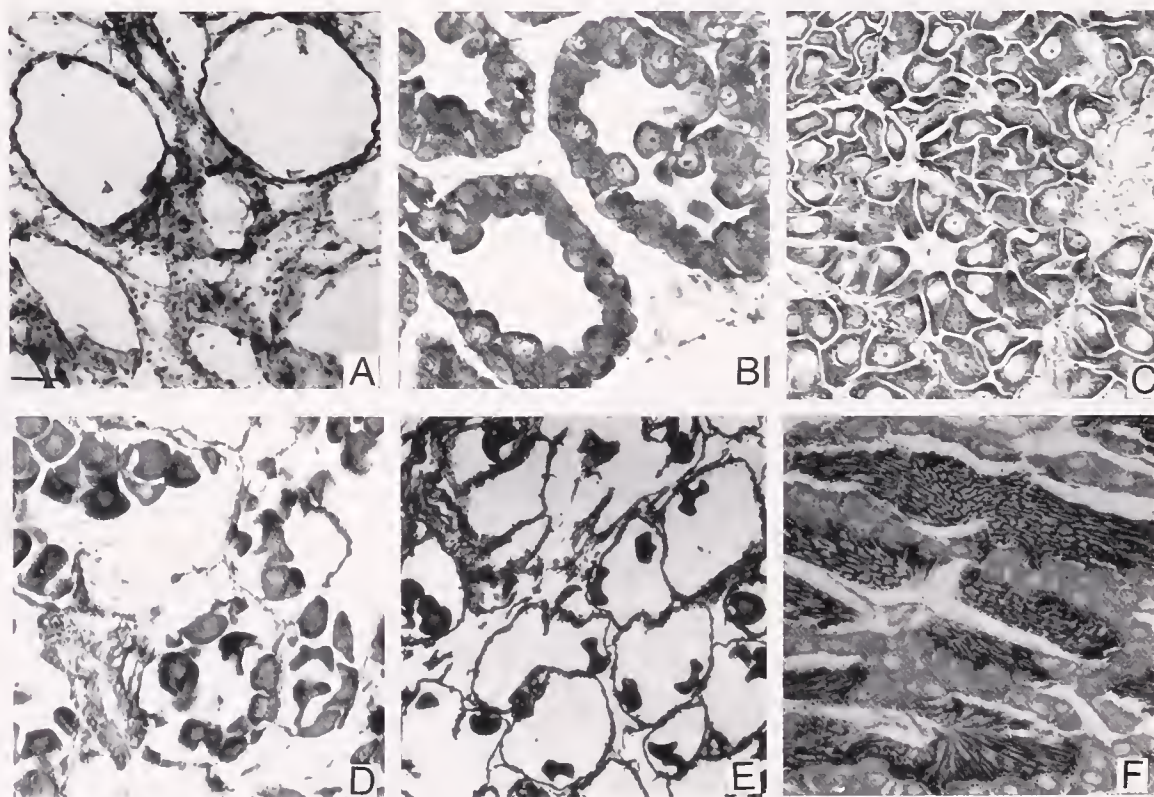


Figure 2. Photomicrographs of gonadal stages of female *S. leucacanthus*: a) indifferent; b) developing; c) ripe; d) spawning; e) spent; f) hermaphrodite; scale = 50 μ m.

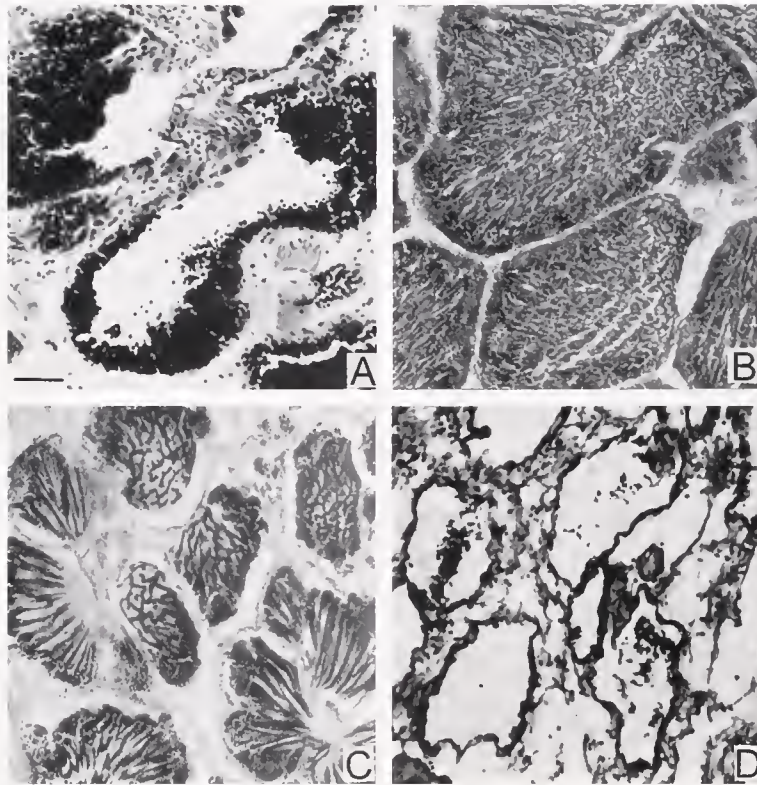


Figure 3. Photomicrographs of gonadal stages of male *S. leucacanthus*; a) developing; b) ripe; c) partially spawned; e) spent; scale = 50 μ m.

occupying the center of follicles were classified as hermaphrodites (Fig. 2F). Size at maturity was estimated by plotting the relative cumulative frequency of spawning organisms against shell height measurements and tracing a line from 50% cumulative frequency of spawning to the shell height measurements.

To obtain the mean size of the oocytes at each sampling date, the diameters of at least 100 oocytes, from six randomly selected females, were measured using an eyepiece graticule calibrated with a stage micrometer. The measurements were made along the longest axis of the oocyte sectioned through the nucleus. Individuals with few measurable oocytes and extensive phagocytosis were not used, following the criteria of Grant and Tyler (1983a) and Grant and Tyler (1983b).

RESULTS

The range in shell height of captured male organisms was from 30 to 120 mm, with the mode at 85 mm. Females had shell heights between 55 and 120 mm, with the mode at 85 mm. The females with spermatozoa in the center of follicles were considered hermaphrodites and had shell heights from 55 to 105 mm, with the mode at 75 mm (Fig. 4). The sexual ratio of 1,128 organisms was 213 (19%) females, 516 (45.7%) males, and 43 (3.8%) hermaphrodites. The remaining (356) were undifferentiated. The sex ratio of the total sample ($X^2 = 125$, $n = 729$) differed significantly ($p < .05$) from the expected ratio of 1:1.

The reproductive cycle of *S. leucacanthus* was remarkably similar over the 2-year study (Fig. 5). From November to March each year the population was inactive, as determined by the presence of spent or indifferent stages. The developing stage started in March and April, and the population was fully ripe from June to August, when spawning began. In November, all the individuals

were spent. Figure 6 shows that the size at spawning in the population of *S. leucacanthus* is 75 mm (shell height); however, individual organisms may start spawning at 40 mm shell height.

Figure 7 shows the monthly mean diameter of intraovarian oocyte measurements of *S. leucacanthus* from April 1994 to October 1995. The mean diameter of mature oocytes is 56.4 μ m (SD = 10 μ m). Monthly measurements from 1994 show that the development of oocytes in the follicles have two periods of growth corresponding to gonad classification (Fig. 7). The first period of growth was from March to June and corresponds with the devel-

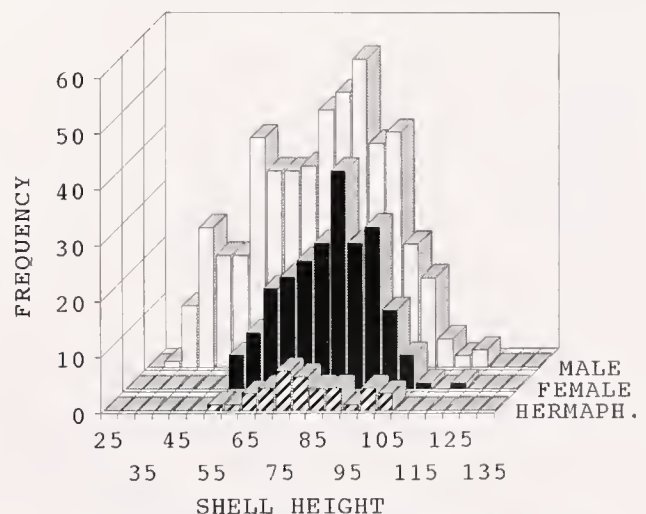


Figure 4. Frequency distribution for 5 mm size classes (shell height) of male, female, and hermaphroditic organisms of *S. leucacanthus*.

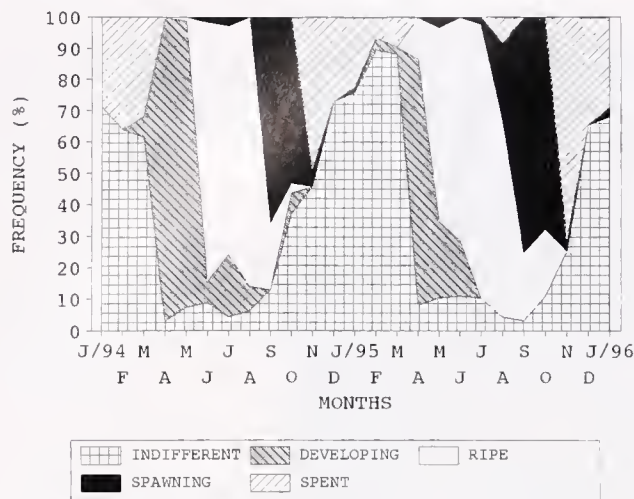


Figure 5. Reproductive cycle of *S. leucacanthus* from January 1994 to January 1996.

oping stage. The second slower growth was from June to September corresponding to maturity of the oocytes. However, in 1995, the oocytes seem to have reached the maximum size in June.

During the study period, the bottom water temperature at Isla Danzante varied from 15 to 26°C. The highest value measured was in November 1994 (25°C), and in September 1995 (26°C). The lowest temperature measured was from January to May in both years investigated (Fig. 8).

The gonad index has a cyclic oscillation (Fig. 9). A single period of maximum value occurred during July in both years investigated, coinciding with the occurrence of highest frequencies of maturity (Fig. 5). The index declined from August to October during spawning. The lowest values measured were from November to February, followed by a period of recuperation from April to June (Fig. 9).

DISCUSSION

The characteristics of gametogenesis and the description of gonadic development stages in *S. leucacanthus* were similar to those described for the pearl oysters *Pinctada mazatlanica* (Gar-

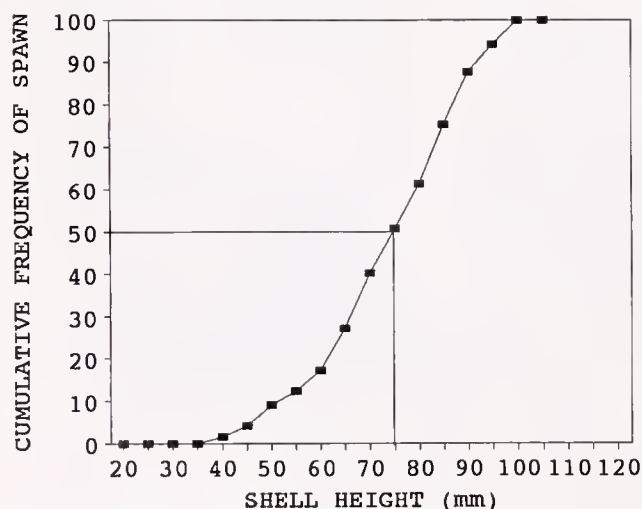


Figure 6. The spawning size of 50% of the population of *S. leucacanthus* from Isla Danzante.

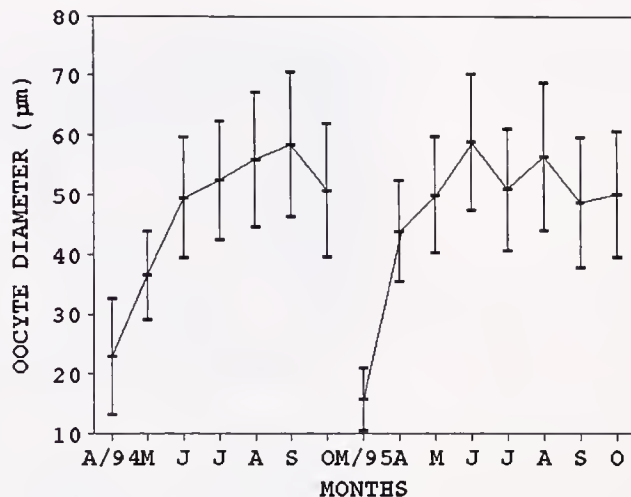


Figure 7. The mean (\pm SD) diameter of oocytes from *S. leucacanthus* from April 1994 to October 1995.

cía-Domínguez et al. 1996), *Pinctada albina* (Tranter 1958a), *P. margaritifera* (Tranter 1958b), *P. fucata* (Tranter 1959), and *P. maxima* (Rose et al. 1990). In *S. leucacanthus*, the gonads increase in weight as the ova and spermatozoa grow during the developing and the ripening stages. The gametes of both sexes were spawned about the same time.

In this species, all females collected during April 1994 and March 1995 were in the development stage and had spermatozoa in the lumen of follicles. Similar gonadic conditions are recorded for *P. albina* (Tranter 1958a), *P. margaritifera* (Tranter 1958c), *P. maxima* (Rose et al. 1990), and *P. mazatlanica* (García-Domínguez et al. 1996). In addition, the predominance of small (<45 mm shell height) male clams and the lack of female or hermaphroditic clams <45 mm shell height suggests that this species may be a protandric hermaphrodite.

There is one major spawning period annually during August to October, so recruitment occurs once each year. The size at spawning in the population is defined as the smallest height at which 50% of females and males sampled are spawning (Somerton 1980). In this study, some individuals exhibit signs of spawning at

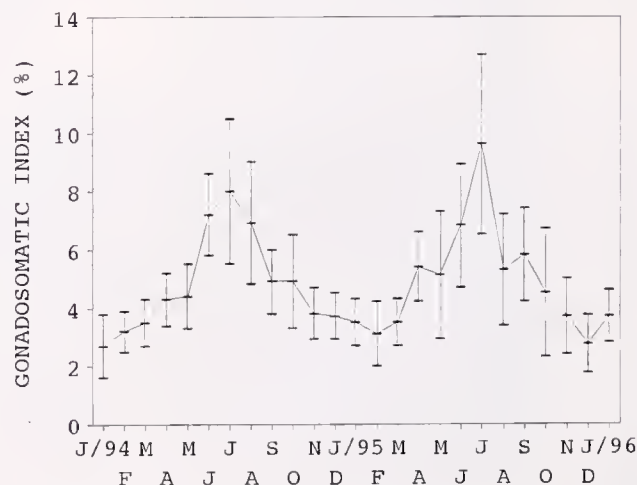


Figure 8. Variation in bottom temperature at Isla Danzante, Gulf of California, and frequency of spawning of *Spondylus leucacanthus* from January 1994 to January 1996.

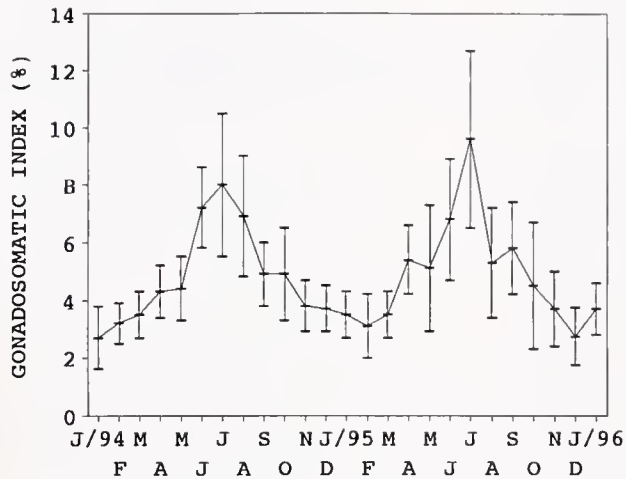


Figure 9. The mean gonad index value \pm SD in *S. leucacanthus* from January 1994 to January 1996.

40 mm shell height; however, the main spawning in population occurs at 75 mm shell height.

The mean diameter of fully ripe oocytes of *S. leucacanthus* is similar to oocytes of *Modiolus capax* (Ochoa-Báez 1985), *Argopecten circularis* (Villalejo-Fuerte and Ochoa-Báez 1993), *P. mazatlanica* (García-Domínguez et al. 1996), *Laevicardium elatum* (Villalejo-Fuerte et al. 1996a), and *Megapitaria squalida* (Villalejo-Fuerte et al. 1996) found in the Gulf of California. When we compare oocyte diameters with gonadal stages in *S. leucacanthus*, it is clear that the minimum diameters coincide with the developing stage and maximum diameters coincide with the ripe and spawning stages. The oocyte diameters are reflective of the gametogenic cycle, similar to *Mercenaria* spp. (Hesselman et al. 1989), *Placopecten magellanicus* (Dibacco et al. 1995), *Glycymeris gi-*

gantea (Villalejo-Fuerte et al. 1995), and *L. elatum* (Villalejo-Fuerte et al. 1996a).

Temperature is an important environmental factor in the regulation of bivalve reproduction (Sastry 1979). The reproductive cycle of *S. leucacanthus* at Isla Danzante shows a clear seasonality related to the bottom water temperature. The inactive period is from November to March with water temperatures decreasing from 25 to 18°C. Gametogenesis occurs at the lowest temperature (18°C), with ripening coinciding with the increasing water temperatures (18 to 23°C) and spawning with the highest values (23 to 26°C). A similar relationship between the temperature and gonadal activity has been observed in such other bivalve species as *Perna picta* (Shafee 1989), *A. circularis* (Villalejo-Fuerte and Ochoa-Báez 1993), *G. gigantea* (Villalejo-Fuerte et al. 1995), and *L. elatum* (Villalejo-Fuerte et al. 1996a).

In *S. leucacanthus*, the mean values of the gonad index are representative of the gonad development and can be associated with the reproductive condition. The lowest values of gonad index occur during the inactive period (November to March), when the gametes are absent. The increase in number and volume of gametes during the development and ripening stages yields the highest values of the gonad index during June to August when the individuals start spawning. A similar relation between this index and the reproductive condition has been observed for other bivalves (Sastry 1979, Dibacco et al. 1995, Villalejo-Fuerte and Ceballos-Vázquez 1996).

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EFFECTS OF BAITWORM DIGGING ON THE SOFT-SHELLED CLAM, *MYA ARENARIA*, IN MAINE: SHELL DAMAGE AND EXPOSURE ON THE SEDIMENT SURFACE

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ABSTRACT Experiments conducted during the fall of 1997 on an intertidal flat in Maine determined the extent of shell damage and exposure of *Mya arenaria* on the sediment surface resulting from commercial bloodworm (*Glycera dibranchiata*) digging. We conservatively estimate that worm diggers dig up and expose on the sediment surface approximately 6% of the greater than 2 mm fraction of the clam population each time they turn over the sediment. Twenty percent of the clams had at least one valve damaged. Fifteen percent of intact clams exposed were found with their siphon up (normal living position), 41% with their siphon down, and 44% were horizontal on the sediment surface. Large clams (5.7 cm average shell length) placed on the sediment surface in the siphon up position reburied faster and to greater depths than those in horizontal or inverted positions. Small clams (2.7 cm shell length) buried faster than large clams, and those placed horizontally or with their siphons up reburied faster than clams placed with their siphons down. We detected no difference in reburial patterns between large clams exposed on undug and recently dug sediment. Our recovery of large clams after 10 days, however, was much greater (91.8%) from undug sediment than dug sediment (59.4%) and we found twice as many clam shells exhibiting evidence of predation in the dug than the undug area. Only about 50% of the small clams were recovered live. Shell damage of recovered dead clams indicated that predators consumed some missing clams. Our results suggest that baitworm digging negatively affects the survival of *Mya arenaria* by directly damaging shells and by exposing clams to increased risk of predation.

KEY WORDS: *Mya arenaria*, *Glycera dibranchiata*, baitworm digging, shell damage, predation

INTRODUCTION

Intertidal sand and mud flats are harvested for infaunal clams and worms by commercial and recreational diggers world wide (Jackson and James 1979, Blake 1979, Cryer et al. 1987, van den Heiligenberg 1987, Brown 1993, Jamieson 1993, Olive 1993, Wallace 1997, Hylleberg et al. 1986). Harvesting usually involves manually turning over the sediment with a hoe or shovel (see van den Heiligenberg 1987, Hall et al. 1990 for discussions of mechanical harvesting). Moderate, animal-mediated sediment disturbance can have large effects on soft-sediment communities (Posey 1987, Peterson 1991, Wilson, 1991, Flach 1992, Ambrose 1993, Hall 1994, Commito et al. 1995a), so the effects of the massive sediment disruption associated with digging for clams and worms has long been of concern (Jackson and James 1979, Cryer et al. 1987, van den Heiligenberg 1987, Brown and Wilson 1997). Studies examining the impact of digging on intertidal soft-sediment systems all show a dramatic impact on the distribution and abundance of many infaunal taxa, with rates of recovery dependent on species' recolonization abilities (Jackson and James 1979, McLusky et al. 1983, Cryer et al. 1987, van den Heiligenberg 1987, Brown and Wilson 1997).

In Maine, three commercially important species co-occur on intertidal mud and sand flats: *Mya arenaria* L. (soft-shelled clam), the polychaete worms *Glycera dibranchiata* Ehlers (bloodworm), and *Nereis virens* Sars (sandworm). Worms are used as bait in recreational fisheries (Brown 1993). In Maine in 1997, soft-shelled clams ranked eighth in landed value among marine species, and both worms together ranked fourteenth (National Marine Fisheries Service Commercial Landings). All three species are harvested manually. Clam and worm diggers often find themselves digging next to each other, and conflicts between the two fisheries date

back to the 1950s (Maine Legislative Research Committee 1957, Wallace per. com.).

Worm harvesting may affect clams in several ways: direct shell damage, displacement below their natural burial depth in the sediment, or exposure on the sediment surface. Harvesting soft-shelled clams with a hoe typically results in the breakage and death of 14 to 20% of the clams (Dow et al. 1954, Glude 1954, Medcof and MacPhail 1964, Robinson and Rowell 1990). Clam hoes in Maine typically have 4 to 5, 15-cm long, tines (Wallace 1997). Worm diggers also use hoes to turn sediment over, but worm hoes usually have more tines (5 to 7 for bloodworm hoes, 5 to 6 for clamworm hoes), and the tines are longer (21 to 22 cm for bloodworm hoes, 34 to 39 cm for sandworm hoes) (Creaser et al. 1983) as compared to clam hoes. There has been no assessment of damage to clams by worm digging.

Turning over the sediment can bury clams deeper than they normally live. When suffocation and exposure are included as sources of mortality, studies indicate a wide range of mortality rates ranging from 2 to 50% (Medcof and MacPhail 1964, Robinson and Rowell 1990). Clam burial experiments clearly show that a clam's survival declines with increased depth below its natural depth in sediment (Glude 1954, Emerson et al. 1990).

Exposure of clams on the sediment surface increases their chances of freezing or becoming desiccated and greatly increases their susceptibility to avian predators during low tide and to demersal fish and crustacean predators during high tide (Medcof and MacPhail 1964). When the anterior edge of *Mya*'s shell is not in contact with the sediment surface, clams cannot rebury efficiently (Emerson et al. 1990). Consequently, a clam's orientation on or near the sediment surface is likely to affect reburial rate profoundly. Reburial rate is also dependent on sediment type (Emerson et al. 1990), but none of the studies examining reburial rate were conducted in the field with natural sediment conditions (Bap-

tist 1955, Pfitzenmeyer and Droebeck 1967, Emerson et al. 1990). Furthermore, sediment compaction on digging tailing is very different than on undug sediment (Ambrose pers. obs.) and might effect rates of reburial.

Only one study (Beal 1996) has addressed the effects of worm digging on *Mya* populations. Beal (1996) examined the effects of bloodworm and clam digging on the survival and growth of cultured and wild *Mya* juveniles (average shell length 12.5 mm). He found that predation effects during the summer masked any effects of digging on the survival of juveniles. Beal's experiment did not address the effects of digging on larger individuals (greater than 35 mm shell length) that have reached a size/depth refuge from most predators (Commuto 1982).

The purpose of our study was to determine the extent of shell damage and exposure of *Mya arenaria* on the sediment surface resulting from commercial bloodworm digging. Factors likely to contribute to mortality that we examined include: shell damage, exposure and orientation on the sediment surface, reburial rates, and depth of reburial. Experiments were conducted on an intertidal mud flat in Maine on undug and recently dug sediment, providing insight into the potential importance of predation on the survival of clams disturbed by digging.

MATERIALS AND METHODS

Study Site

Observations on the impact of bloodworm digging on *Mya arenaria* and reburial experiments were conducted on an intertidal mudflat at the head of Maquoit Bay, Maine (43°55'N Lat., 70°00'W Long.). A tidal range of 3 to 4 meters exposes an intertidal area of about 81 hectares at mean low water (Heinig and Campbell 1992). Our work was conducted in the middle intertidal, where the sediment is coarse silt, with an average grain size of about 30 μ m (Ambrose unpublished data). During our work from September to November 1997, the sediment (1-cm depth) maintained a temperature of 16°C, while air temperature during daytime low tides ranged from 14 to 18°C. Salinity of the incoming water was typically 34 ppt. Maquoit Bay has been highly productive for shellfish (Heinig and Campbell 1992), and both soft-shelled clams and bloodworms are harvested from the flat. During recent years (1994 to 1998), worm diggers have outnumbered clam diggers on the flat, and as many as 40 worm diggers have been observed at one time (Ambrose pers. obs.).

Survey of Undug and Recently Dug Areas

The number, orientation, and shell damage of *Mya* exposed by bloodworm digging was assessed by surveying newly dug areas. Surveys were conducted immediately following commercial digging. Bloodworm diggers turn over sediment methodically, leaving long rows of sediment completely disturbed (see Brown and Wilson 1997 for photograph). The length and average width (at least three measurements) of dug areas were measured to the nearest 5 cm between 19 September and 29 October 1997. We examined 13 recently dug areas for exposed *Mya*. Two people visually searched and lightly probed the sediment surface of each dug area to locate exposed clams. The length (measured to the nearest 0.1 cm with calipers), shell damage (one or two valves damaged), and siphon position (up, down, horizontal) was noted for all exposed clams. We used chi-square analysis to compare observed clam orientations to a distribution of equal numbers of clams in each

orientation that would be expected if orientation of exposed clams was random. The number of clams exposed in each dug area was converted to a number per 1 m² and averaged over all areas surveyed to determine the average density of clams exposed by worm digging.

The density and size frequency of clams in areas that showed no evidence of recent commercial clam or bloodworm digging were determined based on 25, 0.06-m² cores inserted to a depth of about 15 cm (the depth of a hard clay layer below which on this flat clams do not burrow, and worm diggers do not dig). Undug areas were at the same tidal height and often adjacent to dug areas. The contents of each core were sieved in the field through a 2-mm mesh, and clams retained on the sieve were measured. The size frequencies of exposed clams collected from dug sediment and buried clams from undug areas were compared using chi-square analysis to determine if all size classes of clams are equally likely to be exposed by digging.

Reburial Experiments

Reburial experiments were designed to determine the influence of siphon orientation on the rate clams exposed by digging disappeared from the sediment surface and the depth of reburial of these clams. Experiments were conducted separately for large commercial-sized (mean shell length = 5.7 cm, SE = 0.4) and small (mean shell length = 2.7 cm, SE = 0.5) clams.

Large clams, which had been harvested the previous day, were purchased from a retail dealer. Clams were measured with calipers and those with cracked shells or that did not respond to touch by closing their valves were discarded. Two 4 × 4 matrices of 1 m² plots were established on 25 September 1997, one on recently dug sediment and one on sediment showing no evidence of recent digging. Matrices were about 10 m apart and at the same tidal height. The three treatments were: (1) siphon horizontal, (n = 5 replicate plots); (2) siphon vertical and up in the normal living position (n = 6); and (3) siphon vertical and down (n = 5). Siphon-up and siphon-down clams were pushed one-half shell length into the sediment. Clams placed horizontally were pressed slightly into the sediment. The top 2 to 3 cm of the sediment was very soft, so there was minimal sediment compaction. Treatment replicates were randomly assigned to plots. In each plot, 10 clams were spread evenly on the sediment surface. Changes in siphon position, number of clams still visible on the sediment surface, and the condition of clams (shell damage) were recorded after 24 hours, 3 days, and 10 days. After 10 days, each plot was excavated in 3-cm depth intervals and the sediment in each interval was sieved through a 2-mm mesh. Experimental clams could very easily be distinguished from natural clams, because experimental clams had a much lighter shell color as compared to the black shells of natural clams that had resided longer in anoxic sediment. This difference in shell color was obvious even after 10 days.

The number of exposed clams was compared for each time period separately using a one-way analysis of variance (ANOVA). An F-max test (Sokal and Rohlf 1981) revealed no significant (p > .05) differences among variances. When an ANOVA was significant (p < .05), differences among treatment means were compared using a Tukey multiple comparison test. These analyses were conducted separately for the dug and undug site, because lack of replication of dug and undug sites as a variable precluded a two-way ANOVA with replication. We used a 3 × 3, depth × siphon orientation, contingency table to examine the relationship between

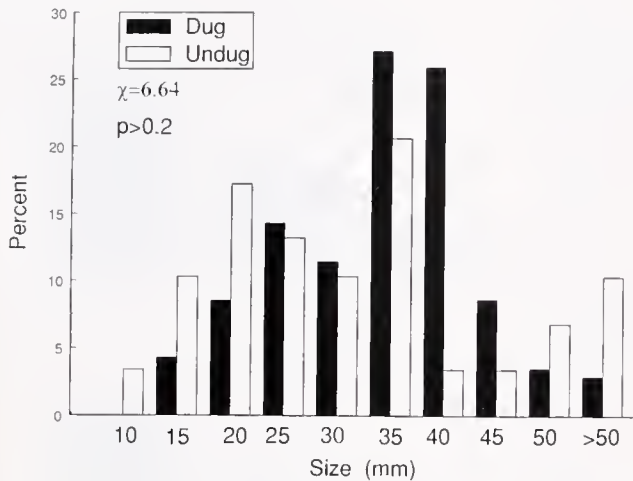


Figure 1. Size frequency distribution of *Mya arenaria* recovered from the sediment surface of recently dug areas ($n = 173$ individuals) and of all individuals sampled with a 0.06-m^2 core ($n = 25$) from undug areas ($n = 29$ individuals) of Maquoit Bay, intertidal flat. There was no significant difference between the two distributions ($\chi^2 = 6.64$, $p > .2$).

siphon orientation and final burial depth. Numbers of clams recovered were compared among treatments using a one-way ANOVA.

Small clams were dug from the flat and held overnight in 20°C , aerated water. Each clam was marked with nail polish to distinguish experimental clams from natural ones. The same experimental design and statistical analyses were used for small clams as for large clams, except that a limited number of small clams only allowed us to establish the matrix on recently dug sediment. The experiment began on 14 October 1997, and sampling, using the same methods used for large clams, occurred 12 h, 24 h, and 3 days later.

RESULTS

Survey of Undug and Recently Dug Areas

A total of 178 m^2 of freshly dug sediment was surveyed in 13 recently dug areas. Dug areas ranged in length from 10 to 25 m (mean = 11.9 m , $\text{SE} = 1.4$) and had an average width of $1.2 \pm 0.15\text{ m}$ (SE), the average arc length of a diggers arm. All diggers were professional bloodworm diggers and were turning over the top 15 cm of sediment.

We found an average of $1.12\text{ clams} \pm 0.19\text{ (SE)/m}^2$ exposed on freshly dug sediment. These clams ranged in length from 1.1 cm to 6.0 cm, and their size frequency distribution was not significantly different from clams sampled by core from nearby undug areas ($\chi^2 = 6.64$, $p > .20$; Fig. 1). In total, 199 clams were collected, and 22.6% of these had at least one valve damaged. This damage was clearly caused by digging, because the breaks were fresh, and in many cases, valves had a hole, probably made from a bloodworm hoe tine. Of the remaining whole clams, 43.7% were found in the horizontal position, 41.1% had their siphon down, and 15.2% were in the normal, siphon up, anterior down, position. This distribution of orientations is significantly different from random ($\chi^2 = 29.9$, $p < .001$). The density of *Mya* in undug areas was $1.16\text{ individuals} \pm 0.24/0.06\text{ m}^2$ or $19.3/\text{m}^2$. Few of these clams were of commercial size ($>5.1\text{ cm}$, Fig. 1).

Large Clam Experiment

A significant difference was found among siphon orientations in the mean number of large clams per plot remaining exposed for each time period for both dug and undug locations (Fig. 2). With the exception of the undug location at 10 days, differences in the mean number of clams exposed among siphon orientations were the same at each sampling and location. Clams beginning the experiment in the normal siphon up position disappeared from the sediment surface faster than clams with their siphons in either the horizontal or down positions. There was no significant difference among these last two orientations. At 10 days in the undug sediment, there was no significant difference in the number of exposed clams between siphon up and horizontal orientations, both of which were different from clams beginning with their siphons down. After 10 days, 19% of the clams remained exposed on the dug site and 24% on the undug site.

More of the clams experimentally exposed were recovered live from undug sediment ($n = 147$, 91.8%) than dug sediment ($n = 95$, 59.4%). Most of the unrecovered clams could not be accounted for, but six dead clams with cracked shells were recovered from the undug site (leaving only seven unaccounted clams) as compared to 13 with cracked shells from the dug site (52 unaccounted clams). No significant difference was found in the mean number of

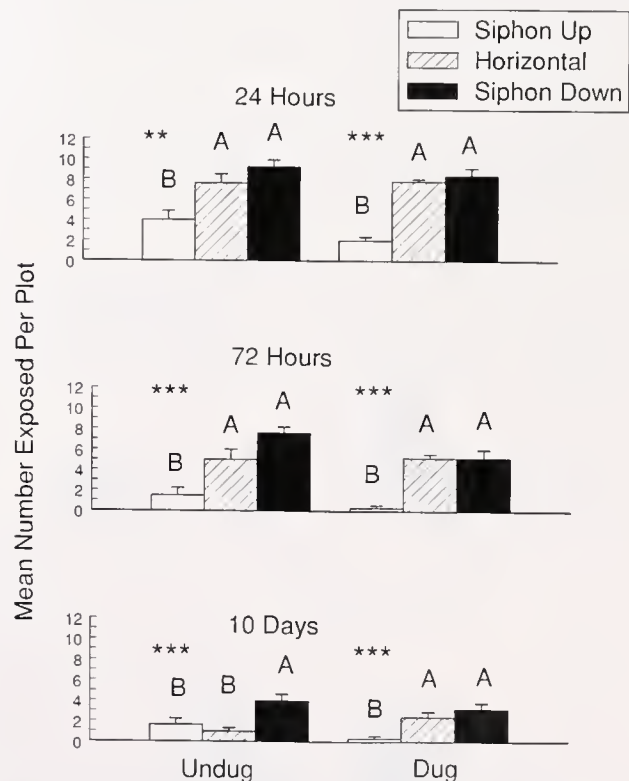


Figure 2. Mean number ($\pm 1\text{ SE}$) per 1 m^2 experimental plot of large *Mya arenaria* remaining exposed on the sediment surface of recently dug and undug sediment after 24 hours, 72 hours, and 10 days. Each plot began with 10 clams. Mean number of clams exposed per plot was compared among siphon orientation (up, horizontal, down) for undug and dug sediment and each time period separately using one-way ANOVA (** = $p < .01$, *** = $p < .001$). Bars with the same letter over them indicate that the means they represent are not significantly different from each other (Tukey multiple comparison test, $p > .05$).

clams per plot recovered from the three treatments from the dug area (up = 6.5, SE = 1.5, down = 5.6, SE = 1.1, horizontal = 5.6, SE = 0.5; $F_{2,13} = 0.21$, $p > .81$). There was, however, a significant difference in the mean number of clams per plot recovered from the undug area (up = 11.3, SE = 0.7, down = 9.6, SE = 1.4, horizontal = 6.2, SE = 0.6; $F_{2,13} = 8.01$, $p < .005$). Significantly ($p < .05$) fewer individuals were recovered from the horizontal treatment than the siphon-up treatment, but there were no other significant differences among treatment means.

For large clams, there was a significant relationship between siphon orientation and the depth in the sediment where clams were recovered after 10 days for both undug ($\chi^2 = 32.49$, $p < .001$) and dug ($\chi^2 = 15.47$, $p < .004$) sites (Fig. 3). A greater number of clams beginning with their siphon in the up position reached a sediment depth of 6 to 9 cm than clams placed on the sediment surface with their siphon down or horizontal. This pattern was the same for dug and undug sediment.

Small Clam Experiment

All small clams had disappeared from the sediment surface by 3 days. Siphon orientation had a significant effect on the mean number of individuals remaining exposed on the surface after 12 and 24 hours (Fig. 4). For both observation periods, no significant difference in the number of exposed clams between siphon-up and siphon-horizontal treatments was found, but significantly fewer clams in these two treatments were exposed than clams placed on the sediment surface with their siphons down.

Approximately half (53.7%) of the 160 small clams were recovered live after 10 days. During the experiment, 2.5% of the clams were recovered dead on the sediment surface. Some of these dead clams had cracked shells, and others had no shell damage and articulated valves. One marked, cracked shell was found 5 m from

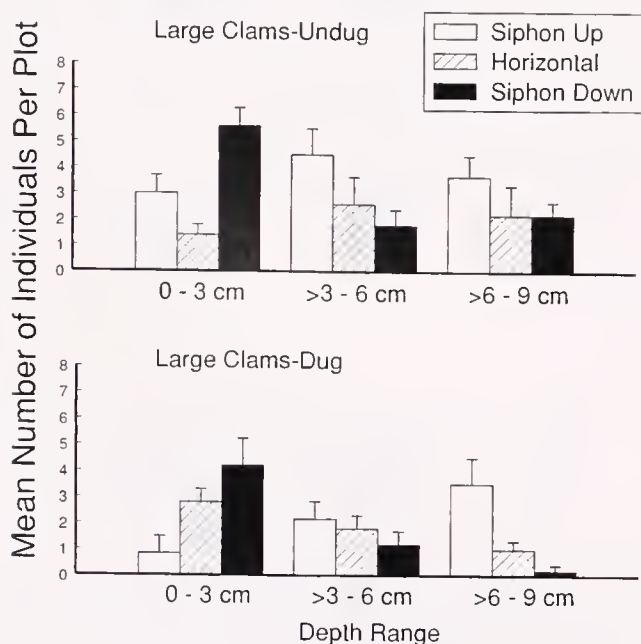


Figure 3. Mean number (+ 1 SE) per 1 m² experimental plot of large *Mya arenaria* with different initial siphon orientations (up, horizontal, down) recovered from three different depth ranges (0 to 3 cm, >3 to 6 cm, >6 to 9 cm) of recently dug and undug sediments. Each plot began with 10 clams.

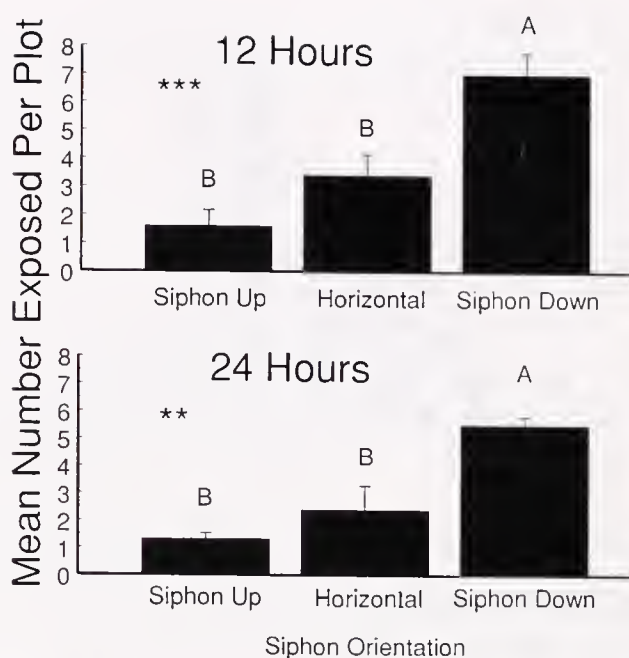


Figure 4. Mean number (+ 1 SE) per 1 m² experimental plot of small *Mya arenaria* remaining exposed on the sediment surface of recently dug sediment after 12 and 24 hours. Each plot began with 10 clams. Mean number of clams exposed per plot was compared among siphon orientation (up, horizontal, down) for each time period separately using one-way ANOVA (** = $p < .01$, *** = $p < .001$). Bars with the same letter over them indicate that the means they represent are not significantly different from each other (Tukey multiple comparison test, $p > .05$).

the experimental matrix. No significant difference in the mean number of clams recovered from the three treatments was found, even though about half as many clams with their siphons oriented down were recovered or compared to those with their siphons up (up = 5.7, SE = 1.0, down = 2.8, SE = 0.4, horizontal 5.2, SE = 1.2; $F_{2,13} = 2.41$, $0.1 < p < .2$). The depth in the sediment at which small clams were recovered was only marginally dependent on their initial siphon orientation (Fig. 5; $\chi^2 = 8.07$, $0.05 < p < .09$). Most individuals were found shallower than 6 cm deep.

DISCUSSION

Our results offer the first experimental evidence that worm digging negatively affects *Mya arenaria* survival by both damaging shells and exposing clams on the sediment surface. Our estimate of shell damage, 22.6%, is similar to the incidence of shell damage reported from clam digging (Dow et al. 1954, Medcof and MacPhail 1964, Robinson and Rowell 1990). Clams with shell damage have less than a 1% chance of survival (Glude 1954), so the shell damage we observed can be assumed fatal. Our estimate of the numbers of clams exposed by digging (1.12/m²) is almost certainly low. Our survey was probably not 100% efficient, particularly for small individuals. In addition, gulls occasionally removed clams before we began surveying.

Clams deposited on the sediment surface by digging can suffer two fates before they rebury: they may die from physical stress (desiccation, freezing, starvation) or predators may consume them. Other researchers (Medcof and MacPhail 1964, Emerson et al. 1990) have remarked that exposure during low tide is often not

fatal. The survival of some large clams on the sediment surface for 10 days (Fig. 2) supports this hypothesis. Death from exposure is certainly more of a problem during the summer and winter when air temperatures can be substantially higher (temperatures higher than 32°C are common) and lower (temperatures below 0°C are common) than those recorded during our fall experiment. Nevertheless, in general, clams on the sediment surface are probably at much greater risk of mortality from predation than from exposure.

The cracked shells we recovered from experimental plots suggest predation by crustaceans and we also observed gulls preying upon clams. Crabs, particularly the green crab (*Carcinus maenas* L.) are major predators on *Mya* in the Northeast United States (Lindsay and Savage 1978, Hidu and Newell 1989, Wallace 1997). Gulls are prominent predators on Maine intertidal flats year round (Ambrose 1986) and have previously been observed to prey on *Mya* (Medcof 1949, Medcof and MacPhail 1964). The nemertean *Cerebratulus lacteus* (Verrill) is also present at the experimental site and can be a voracious predator on *Mya* (Rowell and Woo 1990, Rowell 1992). It cannot be assumed, however, that all missing clams were preyed upon, because *Mya*, particularly small individuals, can move short distances along the sediment surface (Baptist 1995). Waves and currents also transport clams (Emmerson and Grant 1991, Commito et al. 1995b). Nevertheless, unless we assume very different rates of migration or passive transport from dug and undug areas, the large difference in recovery of large clams between dug (59.5%) and undug (91.8%) areas, although not tested statistically, is probably largely a consequence of different levels of predation. Gulls often followed diggers and flocked to dug areas after diggers left and twice as many cracked shells were recovered from the dug than the undug area. Subtidally, predators are attracted to recently trawled areas (Kaiser and Spencer 1994, Kaiser and Spencer 1996, Kaiser and Ramsay 1997, Ramsey et al. 1996, Ramsey et al. 1998). Our results suggest that predators may be attracted to intertidal areas disturbed by baitworm diggers. The lower recovery of small clams (approximately 50%) as compared to large clams is likely a consequence of both greater rates of predation on these sized clams than on large ones and migration or passive transport out of experimental plots. Loss of clams to predation would certainly have been much greater than we observed had we conducted our experiments in the summer when intertidal densities of green crabs are greater than in the fall (Ambrose pers. obs.). In Nova Scotia and New Brunswick, Canada, *Mya* mortality is higher in the summer (August) than winter (February) (Robinson and Rowell 1990).

A clam's risk of predation by epibenthic predators is proportional to the time it is exposed on the sediment surface and the depth to which it reburies (Zwarts and Wanink 1989, Emerson et al. 1990, Zaklan and Ydenberg 1997). Reburial rate is related to siphon orientation (Figs. 2 and 4), and baitworm digging deposits clams on the sediment surface with different orientations. It does not even deposit them equally by siphon orientation, leaving only 15% in a normal siphon-up position. Large clams disappeared from the sediment surface faster when they were placed in an upright position than when in horizontal or down positions (Fig. 2). There was no significant difference among treatments in recovery of clams from the dug area, so this difference is attributable to differences in reburial rate and not to differences in migration or predation among treatments. There was, however, a significant difference in recovery among treatments in the undug area, with an average of more than 10 clams per plot recovered from the upright

treatment and significantly fewer recovered from the horizontal treatment. Clams placed horizontally were possibly most prone to passive transport by waves and currents because their shells were not anchored in the sediment as well as clams in other orientations. Even with this bias, clams still disappeared faster from the upright treatment than from the horizontal treatment (Fig. 2). Large clams with horizontal and inverted orientations have a hard time reaching the sediment with their foot, which is necessary for reburial (Trueman et al. 1966, Emerson et al. 1990). There was no difference in recovery of small clams among treatments and no difference between upright and horizontal treatments in the rate at which clams disappeared from the sediment surface (Fig. 4). Smaller clams in a horizontal position find it easier to reach the sediment surface with their foot than large clams in the same position (Ambrose pers. obs.). Inverted small clams still had a very difficult time reburying.

Large clams placed on the sediment surface with their siphon horizontal or down did not burrow as deeply as those beginning with their siphons up (Fig. 3). Deep-dwelling clams are at a lower risk of being preyed upon by epibenthic predators than are shallower-dwelling individuals (Zwarts and Wanink 1989), so these differences in reburial depths could have consequences for survival. We sampled burial depth after only 10 days, however, and it is possible that clams beginning with their siphons horizontal or down might burrow deeper after a longer time. But even after 6 weeks in muddy sediment, exposed *Mya* reach shallower depths than natural clams (Emerson et al. 1990). Small clams, possibly because they reburrow faster than large clams, did not show a strong relationship between reburial depth and initial siphon orientation (Fig. 5).

Our study may have underestimated reburial rate and, consequently, overestimated mortality if experimental clams were stressed by our manipulations. Two factors might have stressed clams and reduced their reburial rate: (1) clams purchased or drug by ourselves were held 1 day before being placed in the field; and (2) large clams had light shells and, therefore, came from an oxic sediment but were placed in anoxic sediment. The effects of these factors on reburial rate should be considered in future studies.

Most studies of the impact of clam digging on soft-shelled clams have not related harvest effects on clams to the clam popu-

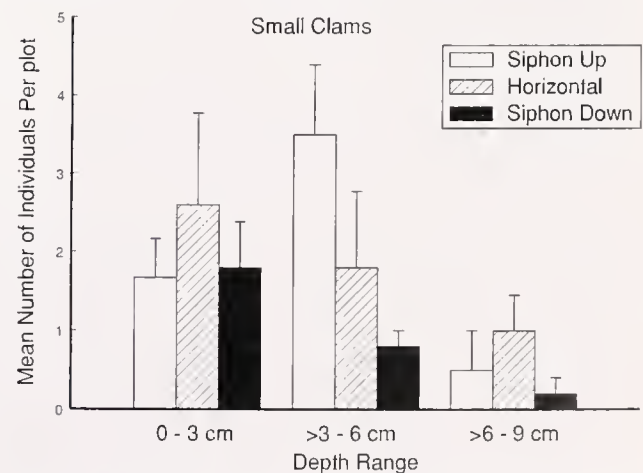


Figure 5. Mean number (± 1 SE) per 1 m² experimental plot of small *Mya arenaria* with different initial siphon orientations (up, horizontal, down) recovered from three different depth ranges (0 to 3 cm, >3 to 6 cm, >6 to 9 cm) of recently dug sediment. Each plot began with 10 clams.

lation (but see Robinson and Rowell 1990). Using our estimates of clam density in areas not recently dug and the density of exposed clams, we estimate that at least 5.8% of the greater than 2 mm fraction of the clam population is exposed each time sediment is dug for worms. The proportion of clams affected by worm digging may vary depending on clam size and density and sediment characteristics. Large clams live deeper in the sediment than small clams (Emerson et al. 1990, Zaklan and Ydenberg 1997, Ambrose unpub. data), and some may live deep enough to avoid bloodworm hoes. At Maquoit Bay, no clams burrowed deeper than the 15 cm necessary to avoid bloodworm hoes, and all sizes were exposed with equal frequency (Fig. 1). If shell breakage as a result of worm digging increases with clam density as it does for clam digging (Dow et al. 1954), the percentage of clams affected by worm digging may be greater in areas with higher clam densities than the relatively low density at Maquoit Bay. Finally, our estimate is for only one turning of the sediment. Worm diggers turn Maquoit Bay over at least three times a year (Ambrose pers. obs.), and some flats are reported to be turned over more (Maine Department of Marine Resources public hearing, April 30, 1998, Wiscasset Maine).

Only one other study has assessed the effects of worm digging on *Mya arenaria*. In a study also done at Maquoit Bay, Beal (1996) found that in the absence of predators, moderate bloodworm digging enhanced juvenile (12.5 mm shell length) survival. Effects of

digging on all sizes of clams, however, need to be determined to assess the impact of baitworm harvesting accurately on *Mya* populations. Effects of worm digging on larger clams are likely influenced by sediment type, clam density, predator type and abundance, season of digging, worm hoe design, and individual digging styles and experience. Clearly, more study is needed before we can fully evaluate the effects of baitworm digging on *Mya* populations. Nevertheless, our results indicate that baitworm digging negatively affects the survival of *Mya* larger than about 2 cm (the smallest experimental clam) and should be considered in their management when both species are present in commercial densities. Furthermore, some of our results can be applied to *Mya* harvesting that leaves behind sublegal-sized clams exposed on the sediment surface and to other harvesting practices that deposit infaunal bivalves on the sediment surface.

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TRANSMISSION ROUTES AND TREATMENT OF BROWN RING DISEASE AFFECTING MANILA CLAMS (*TAPES PHILIPPINARUM*)

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ABSTRACT The potential transmission routes of *Vibrio tapetis*, the causative agent of the brown ring disease affecting manila clams, *Tapes philippinarum*, have been studied under laboratory conditions. The results obtained indicate that the most probable transmission route is by means of direct contact with infected clams. The main factors affecting the incidence of brown ring disease in cultured manila clams were evaluated in normal aquaculture conditions. No significant differences were obtained in regard to clam seed density, but the type of substrate in clam beds significantly affected the disease. Chemotherapeutic treatment of infected clams is proposed, on the basis on the antimicrobial susceptibility of *V. tapetis*, antibiotic solubility in seawater, differential clam toxicity and cost, as being the most adequate treatment the contact of the clams for 1 to 2 h previously to their seeding with nitrofurantoin (10 mg/L seawater for 3 days), flumequine (1.5 mg/L seawater for 3 days), or oxolinic acid (1 mg/L seawater, only one bath treatment).

KEY WORDS: Brown ring disease, *Vibrio tapetis*, manila clams, *Tapes philippinarum*, disease transmission, treatment, epizootic

INTRODUCTION

A disease affecting cultured manila clams (*Tapes philippinarum*) associated with mortalities (about 70%) was first reported in summer 1987 in Brouenou (Finisterre, France) (Flassch 1987). Moribund clams appeared on the clam bed surface before their death, and more than 80% of the affected clams presented a characteristic organic brown deposit on the inner surface of the shell margins, generally located between the pallial line and the edge of the shell, which frequently extended toward the pallial cavity. Because of these typical signs of the disease, it is called brown ring disease (BRD) (Paillard et al. 1989).

BRD appeared in Spain in the summer of 1989 associated with massive mortalities of cultured manila clams of several commercial production areas in southwestern Spain (province of Cadiz). Later, several outbreaks of the disease were recorded in Cantabria (northern Spain), Galicia (northwestern Spain), and Andalusia (provinces of Cadiz and Huelva, southwestern Spain) (Castro et al. 1992, Castro et al. 1993). For this reason, the disease constitutes a major obstacle to expansion of shellfish aquaculture on the Atlantic coasts of France and Spain. Several sanitary measures based on epizootic data have been proposed to decrease this disease, such as the control of BRD incidence in seed lots, reduction of the seeding density, and adaptation of the seeding to optimal season. The use of chemotherapeutics in intertidal shellfish culture zones is impractical because of its high cost and low efficacy. For this, prevention criteria are the only mechanisms to control diseases in the extensive cultures of shellfish (Alderman 1992). Similar prophylactic measures have proved to be effective in the control of other epizootic shellfish diseases, such as bonamiasis and marteliasis in *Ostrea edulis* (Grizel et al. 1986, van Banning 1988), *Perkinsus*

spp. infection, and the MSX disease caused by *Haplosporidium nelsoni* in the case of *Crassostrea virginica* (Andrews and Ray 1988, Ford and Haskin 1988).

The incidence of BRD was previously studied by Castro (1994), who analyzed several manila clam populations seeded on different areas of Cadiz Bay. This study established a characteristic temporal pattern for BRD. The incidence of the disease increased in the first months of culture, achieving a peak in summer or autumn (between 10 and 20% of affected specimens, depending upon the population and zone). In the following months, the incidence of BRD maintained or decreased, but in the second summer of culture, substantial increase in BRD occurred again. The seasonal nature of BRD may be explained by the negative effect that high temperatures produced on the physiology of manila clams (reduction of the growth and increase in the metabolic rate), which provokes a decrease in the defense response of the clams (Fisher et al. 1987, Newell and Barber 1988). In addition, the maximal incidence of the BRD symptoms is coincident with the reproductive period of this clam species on the south Atlantic coast (Morel 1988, Devauchelle 1990, Sarasquete et al. 1990), which corresponds to a drop in the clam condition index.

The causative agent of the BRD has been described as a new bacterial species, designated *Vibrio tapetis* (Borrego et al. 1996). Although several studies have been carried out to determine the antigenic, genetic, and molecular relationships between *V. tapetis* and other pathogenic *Vibrio* species (Castro et al. 1995, Castro et al. 1996, Castro et al. 1997a), little information is available regarding transmission mechanisms and factors that affect the incidence and prevalence of BRD in clam culture. For these reasons, we studied transmission routes of *V. tapetis* as well as factors

TABLE 1.
Conditions in *Vibrio tapetis* transmission experiments.

Tank No.	<i>V. tapetis</i> Dose	Clam Separation
1	2.5×10^{3a}	Yes
2	2.5×10^3	No
3	2.5×10^5	Yes
4	2.5×10^5	No
5	Control	Yes
6	Control	No

^a Colony-forming units (CFU) per clam.

affecting prevalence of the disease. In addition, several antimicrobial treatments were tested, and their efficacy was evaluated.

MATERIALS AND METHODS

Factors Affecting Disease Incidence

Seed Density

The influence of seed density on the prevalence of BRD signs was studied in an immersed cylindrical containers with forced upwelling of the Research Center "El Toruño" (Cadiz, Spain). Manila clam seed (about 10,000 specimens) of 0.2 to 0.5 g were tested previously to detect *V. tapetis*, using the technique described by Castro et al. (1995). Healthy seed were placed in four containers at two different densities: (1) high (600 seeds/m²), and (2) low (200 seeds/m²). The clam seeds were then allowed to grow for 3 months, and sampling extended over the 4th, 5th, and 6th months. About 100 specimens per month per container were analyzed for the following parameters: wet weight, anteroposterior length, BRD signs, shell deformity, and mortality. BRD signs were recorded by examination of the inner surface of the clam shells under a stereomicroscope.

Substrate Types

Two populations of clams from the same stock of seed (wet weight 0.3–0.5 g) without BRD symptoms, were used to study the influence of the substrate type on disease prevalence. Population A (about 800,000 specimens) was seeded on transformed substrate consisting of sand and fine gravel at a density 425 clams per m² and with a coefficient of tide (semidiurnal type) of 0.4 to 0.7. Population B (about 1 million specimens) was seeded on natural

mud substrate at a seed density of 400 clams per m² and with the same coefficient of tide. Seven samples (about 100 clam specimens) of each population were collected during a 13-month period and analyzed for the same parameters as those for the seed density experiments.

BRD Transmission Routes

To establish the potential pathogen transmission routes, (1) direct contact (by contact with clams or sediment) or (2) water route, 600 specimens of *T. philippinarum* (5–7 g wet weight) were experimentally inoculated with *V. tapetis* strain CECT 4600^T, following the methodology described by Castro et al. (1997b). Two doses of *V. tapetis* were used to inoculate the clams: 2.5×10^3 colony forming units (CFU)/clam and 2.5×10^5 CFU/clam.

The transmission experiments were carried out in the research center "Agua del Pino" (Huelva, Spain), using methacrylate tanks with a bed of about 10 cm of autoclaved marine sediment and recirculating water system (36‰ salinity and 24°C). For each pathogen dose, two kinds of tanks were used, one with two compartments separated by a methacrylate sheet from the bottom of the tank to 2 cm below water level and sealed with silicone, and the other without separation (Table 1). In first kind of tank (nos. 1 and 3), 100 labeled injected clams and 100 uninoculated clams were placed in each compartment. In tanks without separation (nos. 2 and 4) the same number ($n = 100$) of labeled injected and non-injected clams were held together. Tanks 5 and 6 contained 100 clams injected with sterile artificial seawater and 100 clams that were not injected. Clams were fed twice a week with 50 mg/tank of lyophilized *Tetrasehuis* spp. After 30 days, the clams were examined for survival and incidence of BRD signs.

Antimicrobial Susceptibility of *V. tapetis*

The antimicrobial susceptibility of *V. tapetis* was evaluated using the disk diffusion technique described by Barry and Thornsberry (1991) using Mueller–Hinton agar no. 2 (BioMerieux) supplemented with NaCl to achieve a final concentration of 2% (w/v). The following antimicrobial agents and concentrations (supplied by BioMerieux) were used: ampicillin (Ap, 10 µg), amikacin (An, 30 µg), streptomycin (Sm, 10 µg), kanamycin (Km, 30 µg), neomycin (Nm, 30 µg), gentamycin (Gm, 10 µg), tobramycin (Tm, 10 µg), oxolinic acid (OA, 2 µg), tetracycline (Tc, 30 µg), oxytetracycline (OT, 30 µg), erythromycin (E, 15 µg), chloramphenicol (Cm, 30 µg), nitrofurantoin (Fm, 300 IU), and flumequine (UB, 30 µg). Twenty-two strains of *V. tapetis* isolated from

TABLE 2.
Growth and pathological parameters of *Tapes philippinarum* seeds cultured at different densities.

Parameters	Low Density (200 Clams/m ²)				High Density (600 Clams/m ²)			
	Dec.	Jan.	Feb.	Mar.	Dec.	Jan.	Feb.	Mar.
Wet weight (g)	0.54	0.58	0.59	0.67	0.54	0.60	0.61	0.50
Length (mm)								
Mean	13.81	14.28	14.60	14.70	13.18	14.40	14.50	14.50
SD	1.92	1.86	1.60	1.96	2.21	1.89	1.26	1.38
BRD signs (%)	4.23	4.34	3.52	2.57	2.54	4.50	ND	5.72
Shell deformity (%)	7.62	2.60	10.13	4.13	13.55	4.51	13.52	11.25
Mortality rate (%)	7.62	ND	1.98	4.50	1.27	1.52	3.88	5.71

ND: No data.

SD: Standard deviation.

different zones in northwestern France (Borrego et al. 1996) were used in these experiments.

Serial dilution of the antimicrobial agents on agar petri dishes (Sahm and Washington 1991) was performed to determine experimentally the minimal inhibitory concentration (MIC) of the antimicrobials assayed. Serial dilutions of the antimicrobial drugs were carried out on Mueller–Hinton agar no. 2 supplemented with 2% NaCl.

To study the potential application of the antimicrobial agents effective against *V. tapetis* as a treatment tool, the following factors were taken into consideration: antibiotic solubility and stability in seawater, and cost. For these assays, the antimicrobial concentrations recommended for aquaculture practices in European Union (Schnick et al. 1997) were used. The toxicity to clams was tested recording the mortality rate of the clams (0.3–0.5 g wet weight) at the end of the treatment period (maximum 3 days). The efficacy of antimicrobial treatments was evaluated on injected clams (10^5 CFU of *V. tapetis* strain CECT 4600^T per clam) treated and nontreated with the antimicrobial tested. After treatment, clams (100 per experimental group) were placed in aquaria with 40 L of 5- μ m filtered seawater and maintained at $19 \pm 1^\circ\text{C}$ with aeration. BRD signs were recorded after a 1-month period, as above mentioned.

RESULTS

Factors Affecting Incidence of the Disease

Results obtained for growth (wet weight and anteroposterior length) and pathological (BRD signs, shell deformity, and mortality rate) parameters in both clam populations tested (low and high seed density) were examined using a one-way analysis of variance (ANOVA) (Table 2). Only in the last sampling (March), were significant differences between the populations (low and high density) obtained for clam length ($p = .0079$), BRD signs ($p = .0468$), and shell deformity ($p = .0158$).

The growth rate of clams in different substrate types was analyzed by wet weight, length, and productivity per m^2 of substrate, which was estimated from the values of density and mean weight of clams. Weight and length of the cultured clams in both substrates was similar throughout the experiment (Table 3), with no significant differences ($p > .01$) between clam populations. However, a significantly lower biomass in natural substrate was recorded ($p = .0145$), largely caused by the higher clam mortality rate (Fig. 1).

Mortality rate was clearly different between clam populations (Fig. 2). In the natural substrate, the mortality increased quickly in

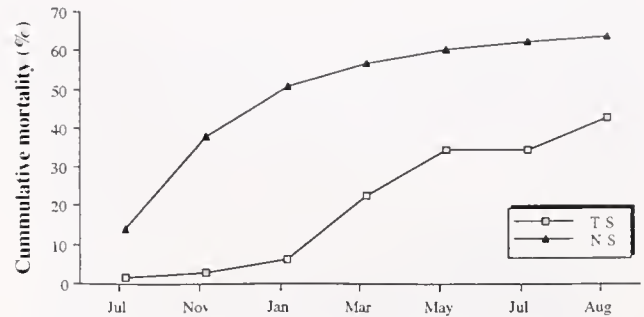


Figure 1. Cumulative mortality of clams cultured in two substrate types (T.S.: transformed substrate; N.S.: natural substrate).

the first months of culture, achieving a cumulative mortality rate of 48.8% after 6 months (January), with a peak of mortality of 27.1% in November. Monthly mortality percentages in subsequent samplings were lower than 13%, with a minimum of 2.6% (Fig. 2-A). At the end of the experiment, cumulative mortalities on natural substrate were 62%. In contrast, low monthly mortality rates were recorded in transformed substrates during the first months of culture (lower than 5%). Higher mortality peaks appeared in spring and summer months 8–13 with mortalities lower than 20% (Fig. 2-B). The cumulative percentage in the transformed substrate was 41.2%, which was significantly lower ($p < .01$) than that obtained for natural substrate (62%).

The percentage of BRD signs during the sampling period varied between 0 and 15% (mean value 6.4%) in transformed substrate (Fig. 2-B), with increases in the percentages in both summers. A similar rate was observed in the natural substrate, varying between 9 and 45%, with a mean value of 19.2% (Fig. 2-A). A chi-square test showed that there was a significant difference ($p < .05$) between substrates for BRD signs. Shell deformity was similar in both substrates (Fig. 2), with high occurrence at the end of the sampling (52% in transformed substrate and 74% in natural substrate).

BRD Transmission Experiments

The incidence of the BRD was dependent on the *V. tapetis* concentration and on separation of tanks (Table 4). No significant differences ($p > .05$) were obtained between the mortality rate of the uninoculated and inoculated clams, although for the experimentally infected clams, the pathogen doses as well as the separation in the tanks significantly affected mortality rate ($p = .013$).

TABLE 3.

Growth of *Tapes philippinarum* in natural and transformed substrates.

Months Sampling	Biomass (Kg/m^2)		Length (mm)		Wet Weight (g)	
	Natural	Transformed	Natural	Transformed	Natural	Transformed
July	0.60	0.75	18.6	19.6	1.7	1.8
November	0.60	0.80	19.6	20.1	2.0	2.2
January	0.65	0.90	20.2	21.8	2.3	2.4
March	0.70	0.90	22.2	22.3	2.5	2.6
May	0.80	1.00	24.0	23.7	4.0	3.2
July	0.80	2.20	25.0	27.0	4.4	4.6
August	0.90	2.30	27.0	29.0	5.7	5.9

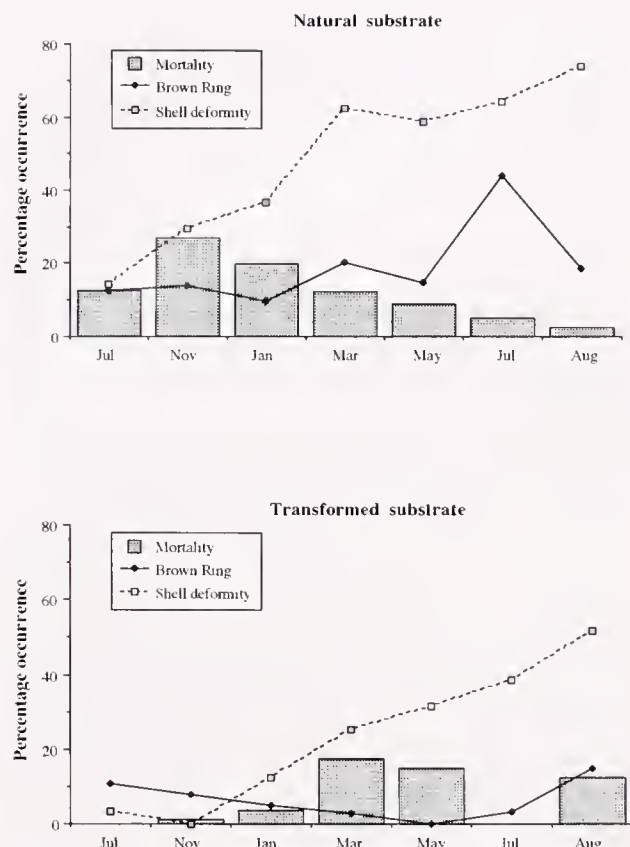


Figure 2. Temporal evolution of the pathological parameters studied in clams seeded in two substrate types (natural and transformed).

Susceptibility to Antimicrobial Agents

The susceptibility of *V. tapetis* to chemotherapeutic agents was established by determining the MICs of the most effective agents against the pathogen. All strains of *V. tapetis* tested ($n = 22$) showed resistance to streptomycin, amikacin, kanamycin, gentamycin, and neomycin. They were sensitive to seven other antimicrobial agents, with MIC ranging from 2 to 4 $\mu\text{g/mL}$ (Table 5). Those antimicrobials belonging to the same antibiotic group showed similar MIC for *V. tapetis*.

To study the potential application of antimicrobials as a treatment tool, the following factors were taken into consideration: antimicrobial MIC for *V. tapetis*, antibiotic solubility and stability in seawater, and cost (Table 5). On the basis of these factors, three

TABLE 5.

Characteristics of the selected antimicrobials effective against *Vibrio tapetis*.

Antimicrobials	MIC ($\mu\text{g/mL}$) Serial Dilution	Seawater Solubility	Cost
Ampicillin	2.0	–	L
Chloramphenicol	4.0	+	H
Erythromycin	4.0	–	H
Flumequine	2.0	+	L
Nitrofurantoin	2.0	+	L
Oxolinic acid	2.0	+	L
Oxytetracycline	2.0	+	H

+: Positive; –: negative; H: high; L: low.

antimicrobials were selected and tested to evaluate their efficacy to prevent the development of BRD. The results showed that the bath treatment was effective to reduce the BRD signs under 7.5 vs. 38% for injected clams without antibiotic treatment, in the following conditions: 10 mg/L for nitrofurantoin (bath during 1–2 h for 3 days), 1.5 mg/L for flumequine (bath during 1–2 h for 3 days), and 1 mg/L for oxolinic acid (only one bath during 2 h). The mortality recorded at the end of the experiment ranged between 2.0 and 8.9% in antibiotic-treated clams, and 8.2% in the nontreated group.

DISCUSSION

In the present study, two substrate types were evaluated in relation to the occurrence of BRD, a natural mud substrate and a transformed (sand and fine gravel) substrate. The results (Fig. 2) demonstrated a higher BRD occurrence and lower clam survival in natural substrate as compared to transformed substrate. These findings confirm the results obtained by Muñoz et al. (1993) and Castro (1994). The physical characteristics of the seeded sediment, as well as the biogeochemical cycle of the organic matter in the substrate, significantly influence the physiology and susceptibility of cultured clams to pathogenic agents. Barillari et al. (1990), in a study carried out in nine sedimentary beds of the Venezia Lagoon, demonstrated that the mortality of *T. philippinarum* was higher in natural substrates of fine particle size than in substrates with greater size particles. In the latter, better circulation of the water and oxygen is produced, avoiding the anoxic process and the macroalgal sedimentation, two parameters that exerted a negative effect on bivalve mollusk growth. Moreover, in fine mud sediments, there is a higher concentration of organic matter (Dahlback and Gunnarsson 1981), which increases the oxygen consumption and

TABLE 4.

BRD prevalence and mortality rate of clams in transmission experiments.

Tank No./Clam Separation/Pathogen Doses ^a	Inoculated Clams		Noninoculated Clams	
	BRD Signs (%)	Mortality (%)	BRD Signs (%)	Mortality (%)
1/Yes/ 2.5×10^3	13	10	8	16
2/No/ 2.5×10^3	17	28	8	18
3/Yes/ 2.5×10^5	24	22	14	16
4/No/ 2.5×10^5	36	28	38	8
5/Yes/Control	0	2	0	6
6/No/Control	2	10	0	9

^a Expressed as colony-forming units (CFU) per clam.

the subsequent growth of anaerobic bacteria with H_2S production (Fenchel and Riedl 1970). The negative effect exerted by the muddy substrata on the clam physiology may also be explained in terms of the energetic costs that require the constant release by the clam of inorganic particulate matter from the sediment. This mechanism involves a weakening of the shellfish that prevents them from responding adequately to external stress.

Epizootic studies on shellfish are based on the population response to a pathogenic agent, but resistance capability to the disease is an individual characteristic. In addition, several environmental factors act as stressing agents, negatively affecting the physiology and defense mechanisms of the host (Sindermann 1983). Therefore, in deficient culture conditions, such as mud substrates, the percentage of highly susceptible clams will be large, and the disease will progress quickly without typical signs until a large proportion of the clam population develops the disease. In contrast, in populations with better physiological conditions, mortality will be associated with advanced steps of the disease and, therefore, there will be a delay between the first signs of the disease and the clam mortality.

Flassch (1989) demonstrated the existence of a close and direct relationship between the seed density and the incidence of the BRD. In the present study, the results obtained (Table 2) do not establish a clear relationship between the seed density and the pathologic parameters tested, except for the last sampling (month of March). Similar results were found by Muñoz et al. (1993) using clam densities of 200 to 600 units/m² for transformed substrate, and 50 to 200 units/m² for natural substrate. In contrast to the results reported by Flassch (1989) in France, the clam seed density does not seem to play an important role in the prevalence of BRD on the Spanish south Atlantic coasts.

The results of the transmission experiments (Table 4) suggest that *V. tapetis* infects manila clams under the laboratory conditions assayed. It seems that direct contact in the sedimentary bed between inoculated and healthy clams enhances the transmission of the infective agent and the development of the disease. Similar results were obtained by Raghukumar and Lande (1988), who experimentally infected rock oyster *Crassostrea cucullata* with the fungus *Ostracoblabe implexa*. This pathogenic microorganism is the causative agent of the "shell disease," and provokes a biodegradation of calcareous substrates of the oyster shells, symptoms similar to those caused by *V. tapetis* both in *T. philippinarum* and *T. decussatus* (Novoa et al. 1998).

The classical practical procedures for controlling the bacteria in juvenile shellfish, on which bacterial populations have established themselves, are based on treatment with dilute solutions of sodium hypochlorite (10 ppm) followed by a thorough seawater rinse (Elston et al. 1982). However, the capacity of seed clams to tolerate

such treatment requires that the valves are intact enough to seal well on closure, and sometimes the above-mentioned treatment causes a higher mortality rate of clams than the pathogenic agent. For this reason, in the present study and according to Sindermann and Lightner (1988), we have evaluated several chemotherapeutic agents on the basis of their efficiency to inhibit *V. tapetis*, their solubility and stability in seawater, their toxicity and accumulation in clams, and applicability and cost.

Few studies have been focused on the antibiotic treatment of shellfish affected by an infectious disease because of the potential risk posed by the fact that drugs may enter the environment as a result of drug released from the treated-animal feces or dissolved in seawater (Ervik et al. 1994). In the present study, 14 chemotherapeutic agents were assayed, and three of them were selected for specific treatment of affected manila clams with BRD. On the basis of the effectiveness of the bath treatment to clam seeds to prevent the development of BRD, the three chemotherapeutic agents selected were nitrofurantoin (10 mg/L seawater for 3 days), flumequine (1.5 mg/L seawater, 1 h, for 3 days), and oxolinic acid (only one bath treatment of 1 mg/L seawater for 2 h). None of antibiotic treatments negatively affect the seed clams (mortality rate lower than 10% and similar to control population) and were very effective to control *V. tapetis*.

The use of antimicrobial drugs as a method to eliminate pathogenic bacteria from shellfish systems has been reported previously (Le Pennec and Prieur 1977, Kraeuter and Castagna 1984). More recently, Brown and Tettelbach (1988) used streptomycin sulfate (50 mg/L) and neomycin sulfate (100 mg/L) treatments against a vibriosis affecting larvae of *Mercenaria mercenaria* and *Crassostrea virginica*. However, there is a danger of resistance associated with the use of these antibiotics, both in naturally occurring *Vibrio anguillarum* (Aoki et al. 1974) as well as other vibrios (Koditschek and Guyre 1974), because R plasmids, which confer resistance to a wide spectrum of antibacterials, has been reported for these *Vibrio* species (Jeffries 1982).

Although the antimicrobial treatments tested in this study have proved to be adequate to prevent BRD, large-scale experiments would be conducted to confirm their applicability in aquaculture facilities. In addition, the treatments proposed must be applied in a controlled system, such as shellfish nurseries, which would avoid the potential risk of the antibiotic dissemination into natural environment.

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GENETIC STUDIES OF THE VENERID CLAM GENUS *KATELYSIA*

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ABSTRACT Two samples of *Katelysia scalarina*, one of *K. rhytiphora*, and one of *K. peronii* were compared for 16 allozyme loci. Diagnostic loci were identified, one of which (SOD*) provided unambiguous discrimination of all three clam species. *Katelysia rhytiphora* and *K. peronii* were found to be the most similar pair ($D = 0.34$). Variability levels were high, with average observed heterozygosities per locus ranging from 0.257 to 0.314, and percentage polymorphism from 62.5 to 81.3. Six samples of *K. scalarina* (three from Tasmania and one each from Victoria, South Australia, and Western Australia), examined for six variable loci, revealed three distinct groups: the three Tasmanian samples; the Victorian and South Australian samples; and the Western Australian sample. Differentiation between the Tasmanian and mainland subpopulations was striking, especially for the PGM* locus. Coastal gene flow mediated by stepping-stone migration between adjacent subpopulations may account for the similarities between subpopulations on the same land mass; whereas, the 10 to 12-day larval period is likely to hinder gene flow across the Bass Strait between Tasmania and the mainland. It is suggested that any future Tasmanian hatchery production should use Tasmanian rather than mainland broodstock, and vice versa, to prevent the introduction to native populations of possibly ill-adapted genotypes.

KEY WORDS: *Katelysia*, clam, allozyme, diagnostic loci, stock-structure, gene flow

INTRODUCTION

The worldwide aquaculture production of clams, cockles, and ark shells is on a par with that of oysters: about 1.25 million tons in 1994, as compared with 1.1 million tons for oysters (FAO 1996). Wild fisheries production is also substantial—in 1992 only about 40% of mollusk production came from aquaculture (FAO 1995). In Australia, mainly wild clam populations are harvested, but the sustainability of this practice is uncertain and so aquaculture production is being considered.

Clams of the genus *Katelysia* are among those most heavily exploited (both as food and fish bait) in the temperate waters of southern Australia. They are some of the most abundant shallow water bivalves in these waters, frequently constituting major or dominant faunal components of soft-bottomed intertidal and shallow subtidal zones of sheltered bays and estuaries (Bellchambers 1998). The three species, *K. scalarina*, *K. rhytiphora*, and *K. peronii*, are frequently sympatric and can be difficult to distinguish morphologically (Roberts 1984, Lamprell and Whitehead 1992). The first two species have very similar distributions, ranging from Western Australia to New South Wales and including Tasmania; whereas, the less abundant *K. peronii* is absent from New South Wales (Nielsen 1963, Lamprell and Whitehead 1992).

In Tasmania, the main clams harvested are *Katelysia* (principally *K. scalarina*) and *Ruditapes largillierii* (thought to have been introduced from New Zealand). Both inhabit similar habitats: *Katelysia* the intertidal and shallow subtidal zone of sheltered bays and estuaries, *Ruditapes* the subtidal zone. The total Tasmanian clam catch rose from 15.56 tons in 1990 and 14.00 t in 1991 to 51.44 t in 1992 and 60.97 t in 1993 (Anon. 1994). From 1994 to 1996, between 23 and 45 t per year of *K. scalarina* alone were harvested (S. Riley pers. comm.). Most are exported live to fish markets in Melbourne and Sydney.

With the increased utilization of the *Katelysia* resource, a hatchery-based industry to supplement or replace the wild fisheries is being considered. Experimental hatchery production in New South Wales of *K. rhytiphora* to metamorphosis has been reported (Nell et al. 1994), and similar work with *K. scalarina* is under way in Tasmania (Kent et al. submitted). In Asia, "almost all of their cockle and clam landings involve some aspect of culture in the grow-out process" (Manzi 1991).

Although there has been considerable ecological research into these species (Nielsen 1963, Coleman 1982, Roberts 1981, Roberts 1984, Bellchambers and Richardson 1995), there have been very few biochemical genetic studies. Nielsen (1963) carried out some preliminary chromatography research but was unable to differentiate *Katelysia scalarina* from *K. rhytiphora*. Roberts (1984) examined protein-stained isoelectric focusing gels, and found some apparent species differences but did not assess within-species variation.

This paper investigates the allozyme genetics of *Katelysia*. The first part examines the genetic relationships of the three taxa, principally to find diagnostic allozyme markers to assist in species identification. The second part examines the genetic population structure of *K. scalarina* in Tasmania and the mainland of Australia, principally to assist fisheries management and any future aquaculture operations. A knowledge of the genetic diversity of wild stocks can help minimize any deleterious effects of introducing hatchery-bred animals or translocating stock (Allendorf and Waples 1996, Rhymer and Simberloff 1996).

MATERIALS AND METHODS

Samples of *Katelysia scalarina* (Fig. 1) were collected from six sites: three in Tasmania and one each in Victoria, South Australia, and Western Australia (Fig. 2, Table 1). Samples of *K. peronii* and



Figure 1. Venerid clams included in this study. From left to right: *Katelysia rhytiphora* (52.7 mm shell length, from Portland Harbour, Victoria; *K. scalarina* (41.2 mm, from Macrae, Port Phillip, Victoria; *K. peronii* (43.7 mm, from St. Kilda, Victoria). Specimens from the Western Australian Museum collection of venerids.

K. rhytiphora (Fig. 1) were collected from the same site in South Australia (Table 1). The clams were transported live to the laboratory in cool-boxes with ice-packs. On arrival, adductor muscles and digestive gland tissues were dissected and stored at -80°C in 2 mL microcentrifuge tubes. The siphon tissue of small individuals was pooled with their adductor muscle tissue.

Morphological Identification

The following descriptions are from Lamprell and Whitehead (1992). The genus *Katelysia* is characterized by subovate to elongate shells with angular posterior; concentric ridges predominating over radial ribs, radial sculpture being weak or absent; pallial sinus medium sized to short, horizontal or ascending; anterior laterals absent. *K. scalarina*: shell length to 40 mm; subovate with angular posterior; close-set, sharp concentric ridges; color white or cream with brown or gray; interior white, often purple posteriorly. *K. rhytiphora*: shell length to 60 mm; ovate to elongate; concentric ridges overlaid by fine radial lirae; color white or light brown, sometimes with dark brown zig-zag pattern; interior white, yellow centrally, purple at muscle scars. *K. peronii*: shell length to 38 mm; ovate and similar to *scalarina*, but finer concentric ridges and posterior less angular; color cream or fawn, sometimes with darker zig-zag patterns; lunule and escutcheon dark; interior purple, sometimes yellow or orange centrally. It should be noted that size varies from site to site: our photographed specimens (Fig. 1) of *Katelysia scalarina* and *K. peronii* are a little larger than the maximum sizes given by Lamprell and Whitehead (1992).

Genetic Analysis

Before electrophoresis, whole tissues were homogenized manually with 2 to 4 drops of distilled water and centrifuged for 5 min at 10,000 rpm; the supernatants were used for electrophoresis (Table 2). Either starch or cellulose acetate gels were used (Table 2). Starch gels used 9% Connaught starch with a discontinuous histidine-citrate buffer system run at 100 V for 4.5 h (gel buffer 0.005M histidine HCl pH 7.0; electrode buffer 0.41M trisodium citrate pH 7.0); the cellulose acetate gels were Helena Titan III plates run at 150 V for 1 h (gel and electrode buffer 0.075 M Tris and 0.025 M citric acid, pH 7.0). Staining techniques followed Richardson et al. (1986) and Hebert and Beaton (1989). Heterozygote banding patterns were consistent with known quaternary structures (Ward et al. 1992).

Sixteen loci were examined in the between-species comparisons (Table 3), and six (see Table 6) were examined in the *Katelysia scalarina* stock-structure analysis. Five of the latter loci were chosen for their variability and their reliability (DIA*, ESTD*, GPI*, MDH-1*, and PGM*), the sixth locus (MDH-2*) was

scored on the same gels as MDH-1*. The MDH patterns immediately enabled any non-*K. scalarina* specimens to be eliminated from the stock-structure analysis.

Where there were multiple loci, the locus encoding the fastest migrating allozyme was designated "1." In the between-species comparisons, alleles were lettered from "a" for the fastest migrating allozyme. In the *Katelysia scalarina* stock-structure analysis, alleles were numbered according to the anodal mobility of their product relative to that of the most common allele observed in the Cockle Creek (TAS2) sample, which was designated "100". (Allele identities between the two studies, where known, are identified in Table 6. Sample sizes were substantially larger for the stock structure analysis; see Table 5.)

Statistical Analysis

For the between-species comparisons, mean sample sizes, numbers of alleles, percent polymorphism (a locus was considered polymorphic if the most common allele had a frequency less than 0.95) and heterozygosities (both observed and unbiased Hardy-Weinberg expected values) were estimated by the BIOSYS-1 package (Swofford and Selander 1981).

For the *K. scalarina* stock-structure analysis, individual sample, and locus tests for goodness-of-fit to Hardy-Weinberg expectations used Fisher's exact test after all but the most common allele had been pooled at each locus. Two genetic diversity parameters were estimated: F_{IS} (the correlation between two uniting alleles relative to the subpopulation and defined as the ratio of the difference between the expected and observed heterozygosities to the expected heterozygosity) and F_{ST} (the correlation between two gametes drawn at random from each subpopulation, defined as the ratio of the difference between the expected total heterozygosity

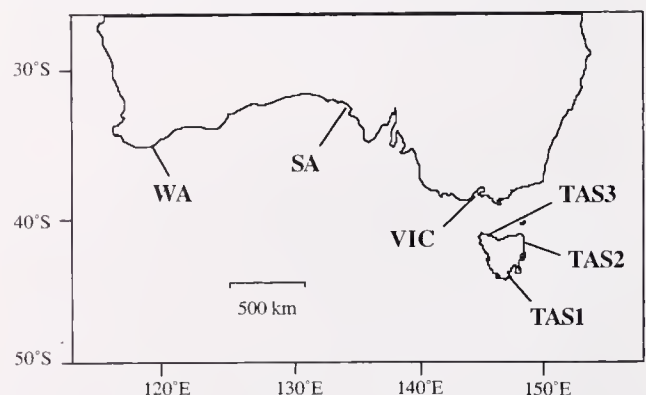


Figure 2. Southern Australia, showing approximate locations of sample sites.

TABLE 1.
Collection details for *Katelysia* species; n = number of clams collected.

Site	State	Abbreviation	Location	Date Collected	n
<i>K. scalarina</i>					
Cockle Creek	Tasmania	TAS1	43°33'S, 146°52'E	Aug. 95	120
Ansons Bay	Tasmania	TAS2	41°03'S, 148°21'E	Sept. 95	120
Smithton	Tasmania	TAS3	40°50'S, 145°00'E	Sept. 95	120
Queenscliff	Victoria	VIC	38°16'S, 144°39'S	Nov. 95	81
Ceduna	South Australia	SA	32°07'S, 133°46'E	Dec. 95	58
Albany	Western Australia	WA	35°01'S, 117°58'E	Dec. 95	110
<i>K. rhytiphora</i>					
Ceduna	South Australia	SA:RHY	32°07'S, 133°46'E	Dec. 95	16
<i>K. peronii</i>					
Ceduna	South Australia	SA:PER	32°07'S, 133°46'E	Dec. 95	16

and the average expected subpopulation heterozygosity to the expected total heterozygosity). F_{IS} essentially estimates deviations from Hardy-Weinberg expectations, and F_{ST} estimates the extent of genetic differentiation of subpopulations. Values of Fisher's exact test, F_{IS} , and F_{ST} were estimated by BIOSYS-1 (Swofford and Selander 1981). Allele frequency homogeneity across samples was tested by the randomised Monte Carlo chi-square procedure of Roff and Bentzen (1989), which obviates the need to pool rare alleles. For each test, 1,000 randomizations of the data were carried out.

For both sets of comparisons, BIOSYS-1 was used to calculate unbiased genetic distances between samples (Nei 1978) and to cluster the resulting distance matrices with the UPGMA (unweighted pair-group method with averaging) algorithm. Sample sizes were smaller for the between-species comparisons, because estimates of genetic distance are far more dependent on numbers of loci than numbers of individuals (Nei 1978, Gorman and Renzi 1979).

When multiple tests of a single hypothesis were carried out, the standard Bonferroni procedure was applied. The p value for a specific test had to be less than, or equal to, $0.05/n$ (where n is the number of tests) to be deemed statistically significant (Miller 1981).

RESULTS

Comparison Between Species

Sixteen loci were examined in four samples, one each of *Katelysia rhytiphora* and *K. peronii* and two of *K. scalarina* (Table 3). Levels of variation in each sample were high, despite quite small sample sizes, with an average of 2.5 to 2.8 alleles per locus and between 62.5 and 81.25% polymorphism. Observed and expected heterozygosities per locus ranged from 0.257 to 0.314 and from 0.283 to 0.377, respectively (Table 4). Sample sizes were too small to warrant Hardy-Weinberg tests.

All species pairs showed diagnostic loci (Ayala and Powell 1972), although only SOD*, which was nearly monomorphic and different in each species, allowed all three species to be discriminated unambiguously. Other diagnostic loci were: *K. scalarina*—*K. rhytiphora*, AK*, APK*, MDH-2*, PGDH*, *K. scalarina*—*K. peronii*, ADA*, AK*, MDH-2*, PGM*; *K. rhytiphora*—*K. peronii*, APK*. Here, a diagnostic locus is defined as one that will enable individuals to be identified with a $\geq 99\%$ chance of correct

identification, given that the two taxa are represented in an unknown sample in equal frequencies.

Katelysia rhytiphora and *K. peronii* were more closely related to one another ($D = 0.337$) than either was to *K. scalarina* (mean $D = 1.047$, range 0.859–1.238) (Table 5, Fig. 3). The within-species genetic distance of *K. scalarina* ($D = 0.068$) was much less than any of the between-species genetic distances (mean $D = 0.905$, range 0.337–1.238) (Table 5, Fig. 3).

Katelysia scalarina Population Structure

Three samples of *Katelysia scalarina* from Tasmania and three from the mainland of Australia were examined to assess population structure (Table 6), both on medium scales (comparisons of Tasmanian samples) and broad scales (comparisons among mainland samples, and of mainland with Tasmanian samples).

Thirty-six tests of Hardy-Weinberg equilibrium ($6 \text{ loci} \times 6$

TABLE 2.
Enzymes used in this study.

Enzyme	EC Number	Locus Abbreviation	Tissue	Gel
Adenosine deaminase	3.5.4.4	ADA*	m	s
Adenylate kinase	2.7.4.3	AK*	m	s
Arginine phosphokinase	2.7.3.3	APK*	m	ca
Aspartate aminotransferase	2.6.1.1	AAT*	m	s
Diaphorase	1.8.1.4	DIA*	d	s
Esterase-D (UV, umb. acetate)	3.1.—.—	ESTD*	m	s
Fumarate hydratase	4.2.1.2	FH*	d	s
Glucose-6-phosphate isomerase	5.3.1.9	GPI*	m	s
Isocitrate dehydrogenase	1.1.1.42	IDHP-1*	d	ca
		IDHP-2*	d	ca
Malate dehydrogenase	1.1.1.37	MDH-1*	m	s
		MDH-2*	m	s
Peptidase (val-leu)	3.4.—.—	PEP*	m	ca
Phosphogluconate dehydrogenase	1.1.1.44	PGDH*	m	ca
Phosphoglucomutase	5.4.2.2	PGM*	d	s
Superoxide dismutase	1.15.1.1	SOD*	d/m	s

Tissue: d = digestive gland (visceral mass), m = adductor muscle. Gel: ca = cellulose acetate, s = starch (see text). Multiple loci encoding for the same enzyme are designated by consecutive numbers, with "1" denoting the fastest migrating system.

TABLE 3.

Allele frequencies at 16 loci in *Katelysia rhytiphora*, *K. peronii*, and two populations of *K. scalarina*; n = number of individuals.

Locus	Allele	<i>K. rhytiphora</i>	<i>K. peronii</i>	<i>K. scalarina</i>	
		SA:RHY	SA:PER	SA	TAS2
ADA*	a	0.031	0.219	—	—
	b	0.219	0.531	—	—
	c	0.063	0.250	0.031	—
	d	0.656	—	0.031	0.333
	e	0.031	—	0.938	0.667
	n	16	16	16	12
AK*	a	0.958	1.000	—	—
	b	0.042	—	—	—
	c	—	—	0.969	1.000
	d	—	—	0.031	—
APK*	n	12	15	16	10
	a	0.036	—	—	—
	b	0.179	—	—	—
	c	0.786	0.042	—	—
	d	—	0.125	0.906	1.000
	e	—	0.833	0.094	—
AAT*	n	14	12	16	12
	a	—	—	0.031	—
	b	0.893	0.367	0.906	1.000
	c	0.107	0.633	0.063	—
DIA*	n	14	15	16	12
	a	0.156	0.219	0.094	0.042
	b	0.594	0.250	0.531	0.333
	c	0.219	0.188	0.281	0.500
	d	—	0.094	—	—
	e	0.031	0.250	0.094	0.125
ESTD*	n	16	16	16	12
	a	—	0.031	0.031	0.167
	b	0.969	0.844	0.969	0.708
	c	0.031	0.063	—	0.125
FH*	d	—	0.063	—	—
	n	16	16	16	12
	a	0.844	0.938	0.594	0.583
	b	0.156	0.063	0.406	0.417
	n	16	16	16	12
	a	—	0.469	—	—
GPI*	b	—	0.094	—	—
	c	0.625	0.438	0.188	0.208
	d	0.125	—	0.344	0.333
	e	0.094	—	0.063	0.333
	f	0.094	—	0.406	0.042
	g	0.063	—	—	0.083
IDHP-1*	n	16	16	16	12
	a	—	—	0.063	—
	b	—	0.083	0.250	0.042
	c	1.000	0.667	0.656	0.875
IDPH-2*	d	—	0.250	0.031	0.083
	n	14	12	16	12
	a	0.036	—	0.063	—
	b	0.929	0.531	0.125	—
MDH-1*	c	0.036	0.031	0.813	1.000
	d	—	0.438	—	—
	n	14	16	16	12
	a	—	0.031	0.031	0.045
MDH-2*	b	1.000	0.969	0.031	0.227
	c	—	—	0.938	0.727
	n	16	16	16	11
	a	0.094	—	—	—
	b	0.906	1.000	0.063	0.042

TABLE 3.

continued

Locus	Allele	<i>K. rhytiphora</i>	<i>K. peronii</i>	<i>K. scalarina</i>	
		SA:RHY	SA:PER	SA	TAS2
PEP*	c	—	—	0.938	0.958
	n	16	16	16	12
	a	—	—	—	0.208
	b	—	—	0.219	0.417
	c	0.179	0.031	0.281	0.292
	d	0.786	0.438	0.438	0.083
PGDH*	e	0.036	0.469	0.063	—
	f	—	0.063	—	—
	n	14	16	16	12
	a	0.107	—	—	—
	b	0.250	0.292	—	—
	c	0.643	0.667	0.083	0.042
PGM*	d	—	0.042	0.917	0.792
	e	—	—	—	0.125
	f	—	—	—	0.042
	n	14	12	12	12
	a	0.031	—	—	0.417
	b	0.219	—	0.156	0.458
SOD*	c	0.563	—	0.813	0.125
	d	0.188	0.438	0.031	—
	e	—	0.531	—	—
	f	—	0.031	—	—
	n	16	16	16	12
	a	—	0.031	—	—
	b	—	0.969	—	—
	c	1.000	—	—	—
	d	—	—	0.969	1.000
	e	—	—	0.031	—
	n	15	16	16	12

subpopulations) were performed (data not shown). Two had P values less than .05 (GPI* at TAS3, $p = .030$, and DIA* at SA, $p = .013$), but neither was significant after Bonferroni corrections for 36 tests. Six tests (one per locus) of whether the average value of F_{IS} was significantly different from zero were performed (Table 7); one was significant after Bonferroni corrections. This was for DIA*, and at 0.177 ($p = .003$) indicated an over-all homozygote excess.

All loci showed significant heterogeneity in allele frequencies ($p \leq .005$) across the six subpopulations (Table 7). For five loci—DIA*, MDH-1*, MDH-2*, GPI*, and ESTD*—the extent of differentiation, although significant, was low, with F_{ST} values of about 0.05 or less. For one locus, PGM*, the extent of differentiation was much more extensive ($F_{ST} = 0.298$). At this locus, nearly 30% of the allele frequency variation could be attributed to differentiation among subpopulations, and there was very little overlap in the allele frequencies of the Tasmanian and mainland subpopulations. The two common alleles in Tasmania (PGM*120 and PGM*100) were uncommon on the mainland, and the two common mainland alleles (PGM*85 and PGM*75) were uncommon in Tasmania (Table 6).

Comparing the three Tasmanian subpopulations with one another found only DIA* to show evidence of spatial differentiation ($p = .038$), but this result became nonsignificant after Bonferroni corrections for six tests. Only one of the 18 pairwise subpopulation tests gave a p value less than .05 (DIA*, TAS2-TAS3, $p = .047$).

TABLE 4.

Summary of genetic variability values at 16 loci in *Katelysia rhytiphora*, *K. peronii*, and two subpopulations of *K. scalarina*.

Locus	<i>K. rhytiphora</i> SA:RHY	<i>K. peronii</i> SA:PER	<i>K. scalarina</i>	
			SA	TAS2
Mean sample size per locus	14.9 ± 0.3	15.1 ± 0.4	15.8 ± 0.3	11.3 ± 0.7
Mean number of alleles per locus	2.7 ± 0.3	2.8 ± 0.3	2.8 ± 0.2	2.5 ± 0.3
Polymorphism % (0.95)	68.75	75.00	81.25	62.50
Observed heterozygosity per locus	0.291 ± 0.059	0.314 ± 0.063	0.276 ± 0.059	0.257 ± 0.070
Expected heterozygosity per locus ^a	0.283 ± 0.058	0.377 ± 0.065	0.299 ± 0.059	0.333 ± 0.071

^a Unbiased estimate (Nei 1977).

a result clearly nonsignificant after Bonferroni corrections. Thus, no significant heterogeneity was observed among the Tasmanian subpopulations.

The three mainland populations showed more differentiation. Three loci—DIA*, MDH-2*, and PGM*—showed evidence of spatial differences in allele frequencies, and all remained significant after Bonferroni corrections ($p = .001$, $.006$, and $.002$, respectively). The three mainland populations were compared pairwise for these three loci. This showed that the Western Australia subpopulation was the most distinct: all comparisons with $p < .05$ included WA (DIA*, WA-VIC, $p = .027$, WA-SA, $p = .014$; MDH-2*, WA-VIC, $p = .003$; PGM*, WA-VIC, $p = .010$, WA-SA, $p = .001$). In none of these pairwise tests was the VIC-SA comparison significant.

Clustering the pairwise subpopulation genetic distances over all six loci (Fig. 4) confirmed these general findings. The three Tasmanian subpopulations clustered together (mean $D = 0.001$) and away from the mainland subpopulations (mean D of Tasmanian vs. mainland comparisons = 0.146 , $n = 9$). Of the mainland sites, the SA and VIC subpopulations were genetically very similar ($D = <0.001$), with the WA subpopulation more distinct (mean $D = 0.003$, $n = 2$).

DISCUSSION

All three species of *Katelysia* are genetically highly variable. Sixteen loci were examined. The average observed heterozygosities per locus ranged from 0.257 to 0.314, and percent polymorphism from 62.5 to 81.3 (Table 4). The average heterozygosity for mollusks, assessed from 105 species and an average of nearly 22 loci per species, is about 0.145 (Ward et al. 1992).

The Nei genetic distance estimates between the different species pairs ranged from 0.337 to 1.238, with the corresponding identity values ranging from 0.714 to 0.290 (Table 5). These val-

ues fall within the typical invertebrate between-species distance range of about 0.20 to 1.60, corresponding to an identity range of about 0.80 to 0.20 (Thorpe 1983). *Katelysia rhytiphora* and *K. peronii* are the most closely related species-pair, a conclusion reached earlier by Roberts (1984) from comparing protein-stained isoelectric focusing gels of the three species. These two species, with $D = 0.337$ and $I = 0.714$, are unusually closely related, although some bivalve (oyster) species show similarly limited divergence (e.g., three *Saccostrea* species, $D = 0.171$ to 0.454 , Buroker et al. 1979). Congeneric clam species typically have D values around 1.0 or greater (e.g., three *Ruditapes* species, $D = 1.050$ to 1.840 , Borsa and Thiriot-Quievreux 1990; two *Chamelea* species, $D = 1.138$, Backeljau et al. 1994), similar to the values of *K. rhytiphora* and *K. peronii* to *K. scalarina* (c. 0.90 and 1.20, respectively).

Diagnostic allozyme loci were identified to enable unambiguous species identification—knowledge that proved useful for distinguishing several *K. rhytiphora* in the putative *K. scalarina* samples. Although sample sizes in the between-species study were quite small, the overlaps in allele frequencies at the diagnostic loci are very small, and the loci are likely to remain diagnostic, at least at the 99% level, in larger samples.

The data were examined for the possible occurrence of between-species hybridization. This seemed possible, because the samples of *Katelysia rhytiphora*, *K. peronii*, and one of the *K. scalarina* samples were sympatric, and the spawning periods, at least of *K. rhytiphora* and *K. scalarina*, can overlap (Nielsen 1963, Roberts 1984). However, in the between-species study, no heterozygotes of the apposite hybrid F1 genotypes were observed for the diagnostic SOD* locus, although sample sizes were small, and rare hybrids would not have been detected.

In the *Katelysia scalarina* stock-structure analysis, which used

TABLE 5.

Unbiased genetic distance (D) (above diagonal) and identity (I) values (below diagonal) (Nei, 1978) between *Katelysia rhytiphora*, *K. peronii*, and two subpopulations of *K. scalarina*.

	<i>K. rhytiphora</i> SA:RHY	<i>K. peronii</i> SA:PER	<i>K. scalarina</i>	
			SA	TAS2
SA:RHY	—	0.337	0.859	0.919
SA:PER	0.714	—	1.173	1.238
SA	0.424	0.309	—	0.068
TAS2	0.399	0.290	0.934	—

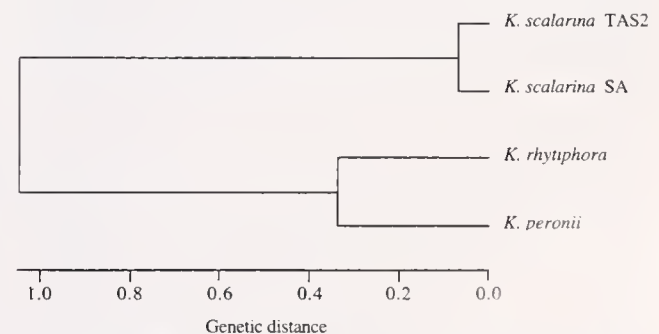


Figure 3. UPGMA cluster analysis of Nei's (1978) unbiased genetic distance (D) among three species of *Katelysia*, based on 16 allozyme loci.

TABLE 6.
Allele frequencies at six loci in six populations of *K. scalarina*.

Locus	Allele	TAS1	TAS2	TAS3	VIC	SA	WA
DIA*	125	—	—	0.004	0.025	—	—
	120 (a)	0.103	0.092	0.081	0.075	0.107	0.051
	110 (b)	0.310	0.298	0.275	0.342	0.286	0.280
	100 (c)	0.413	0.447	0.449	0.308	0.429	0.364
	90 (e)	0.136	0.158	0.140	0.217	0.161	0.178
	75	0.022	0.004	0.051	0.033	0.018	0.121
	60	0.016	—	—	—	—	0.005
	n	92	114	118	60	56	107
ESTD*	150	—	—	0.008	0.019	0.009	0.005
	120 (a)	0.183	0.157	0.171	0.179	0.155	0.141
	100 (b)	0.731	0.712	0.729	0.790	0.836	0.836
	65 (c)	0.075	0.127	0.083	0.012	—	0.018
	50	0.011	0.004	0.008	—	—	—
	n	93	118	120	81	58	110
GPI*	145	0.082	0.065	0.060	0.050	0.018	0.047
	125 (c)	0.212	0.178	0.282	0.094	0.140	0.142
	100 (d)	0.321	0.300	0.312	0.331	0.333	0.392
	80 (e)	0.228	0.252	0.192	0.206	0.167	0.151
	55 (f)	0.114	0.152	0.124	0.294	0.316	0.255
	35 (g)	0.043	0.048	0.030	0.025	0.026	0.014
	n	92	115	117	80	57	106
MDH-1*	165	0.063	0.064	0.042	0.012	0.035	0.037
	135 (b)	0.195	0.182	0.229	0.056	0.053	0.023
	100 (c)	0.742	0.754	0.729	0.926	0.912	0.936
	65	—	—	—	0.006	—	0.005
	n	95	118	120	81	57	109
MDH-2*	140	—	—	—	—	—	0.005
	120 (b)	—	0.008	0.004	0.044	0.009	—
	100 (c)	0.995	0.992	0.992	0.956	0.991	0.991
	70	0.005	—	0.004	—	—	0.005
	n	93	118	120	80	57	107
PGM*	125	0.011	0.008	0.004	—	—	—
	120 (a)	0.317	0.314	0.383	0.006	0.035	—
	100 (b)	0.548	0.610	0.533	0.086	0.096	0.046
	85 (c)	0.091	0.059	0.075	0.790	0.728	0.713
	75 (d)	0.032	0.004	0.004	0.111	0.140	0.222
	55	—	0.004	—	0.006	—	0.019
	n	93	118	120	81	57	108

n = number of individuals.

Allele homologies with Table 3, where known, are in parentheses.

much larger sample sizes, there was evidence of possible F1 hybrids between *K. scalarina* and *K. rhytiphora*. Two of the three Tasmanian animals that were MDH-2*b/c heterozygotes were also MDH-1*b/c heterozygotes (one in TAS2 and one in TAS3)—an unlikely chance occurrence given the low frequency of MDH-1*b/c heterozygotes. In Tasmania, the expected frequency of such a double heterozygote in *K. scalarina* would be about 0.2%, only about one-third the observed frequency of 0.7%. Possibly one or both these animals were F1 hybrids between *K. rhytiphora* and *K. scalarina*, despite the failure of laboratory experiments to produce such hybrids (Nielsen 1963). No such evidence for hybridization was recorded for the mainland subpopulations—none of the six MDH-2*b/c heterozygotes observed in the VIC sample nor the single SA MDH-2*b/c heterozygote was an MDH-1*b/c heterozygote.

The stock-structure analysis of *Katelsia scalarina* revealed three distinct genetic groups: the three Tasmanian samples; the Victorian and South Australian samples; and the Western Australia

sample. The lack of significant differentiation, at least at the scale of resolution afforded by this study, between the three well-separated Tasmanian subpopulations (and between the Victorian and South Australian subpopulations) suggests significant levels of coastal gene flow. On the other hand, the sizeable differences between the Tasmanian and mainland subpopulations, especially at the PGM* locus, suggest minimal gene flow across Bass Strait. The differences at the PGM* locus seemed so large that initially the hypothesis of two unrecognized and allopatric sibling species was entertained, but the Nei genetic distance between the SA and TAS2 subpopulations, 0.068 over the 16 loci, suggests a level of differentiation more typical of within- than between-species differentiation (Thorpe 1983). Similarly, the limited divergence evident in the Western Australia sample does not support the existence of a western Australian subspecies, *Katelsia scalarina polita*, as proposed by Nielsen (1963).

Coastal gene flow mediated by stepping-stone migration between adjacent subpopulations probably accounts for the similar-

TABLE 7.

Genetic diversity statistics for six loci and six populations of *Katelysia scalarina*.

Locus	F_{IS}	p	F_{ST}	p
DIA*	0.177	0.003	0.008	<.001
MDH-1*	0.030	0.575	0.053	<.001
MDH-2*	-0.027	0.090	0.015	.005
GPI*	0.009	0.692	0.017	<.001
ESTD*	-0.030	0.195	0.013	<.001
PGM*	0.043	0.217	0.298	<.001

F_{ST} data analyzed by *t*-tests of individual subpopulations against expected value of 0.

F_{ST} data analyzed by Monte-Carlo chi-square tests of allele frequency homogeneity.

ties between subpopulations on the same land mass; whereas, the short larval life of the species (only c. 10 to 12 days; *Katelysia scalarina*, Kent et al. submitted; *K. rhytiphora*, Nell et al. 1994) is likely to restrict gene flow severely across the Bass Strait between Tasmania and the mainland. Gene flow between the WA and SA subpopulations might be hindered by the intervening presence of long sections of exposed coastline dominated by cliffs and without sheltered bays; these may have fragmented possible clam habitats between these two sampled localities.

The pattern of population differentiation in *Katelysia scalarina* is broadly similar to that seen in other marine clams. For example, populations of giant clams (*Tridacna gigas*) on the same reef, even on extensive reefs such as the Great Barrier Reef, show little differentiation at seven polymorphic loci (mean D c. 0.001, F_{ST} c. 0–0.01); whereas, regionally separated populations are far more divergent (mean D c. 0.069, F_{ST} c. 0.15, Benzie and Williams 1995). The commercial hard clam or quahog of the North American Atlantic coast, *Merccenaria mercenaria*, shows little differentiation from Florida to Massachusetts, but in the north of its range (New Brunswick and Nova Scotia), where populations are disjunct, differentiation is more marked. Comparison of Massachusetts, Maine, Prince Edward Island, and New Brunswick populations gave an F_{ST} estimate for seven polymorphic loci of 0.050 (Dillon and Manzi 1992), only a little less than the mean of 0.067 observed over the six polymorphic loci in our study.

What are the implications of these results for the clam industry? The lack of significant differentiation among the Tasmanian subpopulations suggests that these can be managed as a single stock, although the standard caveats must be made that it takes only a little gene flow to render subpopulations effectively panmictic, and that larger sample sizes and more loci might reveal heterogeneity. A second point is that the very low levels of gene flow between Tasmanian and mainland subpopulations, indicated by their sub-

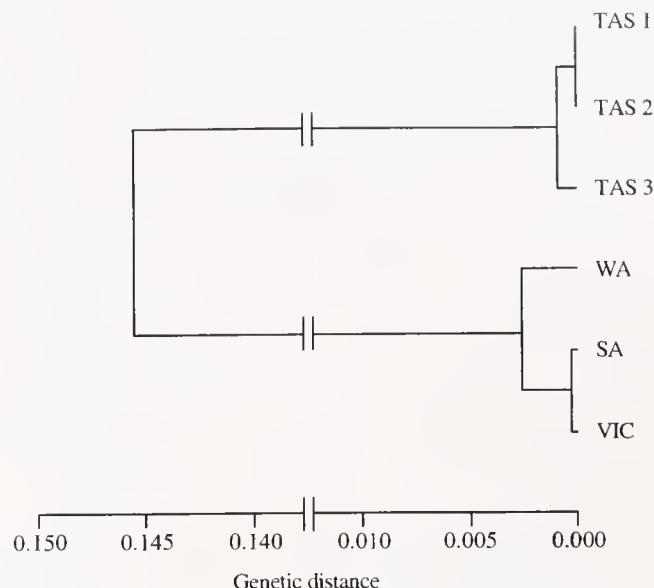


Figure 4. UPGMA cluster analysis of Nei's (1978) unbiased genetic distance (D) among six populations of *Katelysia scalarina*, based on six allozyme loci.

stantial allozyme differences, would facilitate regional co-adaptation of genes and gene complexes. If this scenario is correct, then any Tasmanian hatchery production would be advised to use Tasmanian rather than mainland animals as broodstock, and vice versa. This would help to prevent the introduction of possibly ill-adapted genotypes into the wild via farm escapes. Similarly, any stock enhancement programs that may be embarked on in the future would be wise to use local broodstock to maintain biodiversity levels.

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BROODSTOCK CONDITIONING, SPAWNING INDUCTION, AND LARVAL REARING OF THE STEPPED VENERID, *KATELYSIA SCALARINA* (LAMARK 1818)

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ABSTRACT In a series of trials, broodstock venerid clams *Katelysia scalarina* were conditioned, or obtained directly from fishing grounds, and stimulated to spawn using thermal cycling. Broodstock, initially with only moderately developed gonad, were sufficiently conditioned for mass spawnings after 8 weeks at 13°C in an indoor recirculating seawater system. Ripe wild broodstock maintained at 20°C could still be spawned after 6 weeks in a similar system. Mean fecundity from mass spawning trials ranged between 0.7 and 2.4×10^6 eggs/female, and fecundity estimates for strip spawned individuals varied from 0.3 to 2.2×10^6 eggs/female. Neither intramuscular serotonin injections (6×10^{-8} to 1×10^{-6} mol, total dose), nor strip spawning were reliable methods for producing significant numbers of healthy larvae. Eggs ($69 \pm 2 \mu\text{m}$) developed to D veliger larvae ($110 \pm 1.3 \mu\text{m}$) within 48 h at 20°C. Metamorphosis to spat ($210.9 \pm 2.1 \mu\text{m}$) was observed from day 20 following treatment with 10^{-4} M norepinephrine for 60 min on day 19. Larval rearing was not always successful because of bacterial and viral problems and the extended settlement period for this species.

KEY WORDS: Clam, venerid, *Katelysia scalarina*, broodstock conditioning, spawning, larval rearing

INTRODUCTION

The stepped venerid, *Katelysia scalarina* (Lamarck 1818) is a relatively small clam of 35 to 50 mm shell length (Lush 1992). *K. scalarina* is a shallow (2–4 cm) burrower (Nielsen 1963, Bell-chambers and Richardson 1995) and is found throughout both sheltered and moderately exposed intertidal waters of Tasmania and southern mainland Australia (Gabriel and Macpherson 1962). *K. scalarina* may be distinguished from another closely related species *K. rhytiphora* (Lamy 1937) by the absence of radiating striae and more oblique shape (Gabriel and Macpherson 1962). In addition, *K. scalarina* is often distributed higher up the intertidal zone than *K. rhytiphora* (Roberts 1984).

In Tasmania, *K. scalarina* is the major species in a small clam fishery and is exported to mainland niche markets and occasionally overseas (Treadwell et al. 1992, Zacharin 1993). In recent years, concerns over a decrease in abundance of this species in some areas have necessitated a reduction in catch quotas and given impetus to research into the feasibility of producing hatchery-reared stock for culture or fisheries enhancement.

Currently, there is no commercial aquaculture production of *Katelysia* spp. within Australia, although research into hatchery production and growout of *K. rhytiphora* has been undertaken (Nell et al. 1994, Paterson and Nell 1997). Although oyster hatchery techniques have much in common with clam culture (Maguire 1992), and in the United States have required little modification for clam production (Castagna and Manzi 1989), further investigation into hatchery techniques for temperate clams, including these endemic species, is still required (Maguire 1992, Nell et al. 1995). Potential bottlenecks exist with supply of broodstock, spawning,

larval rearing, and metamorphosis, and this study addresses aspects of these topics for *K. scalarina*.

MATERIALS AND METHODS

All clams collected from natural habitats were transported to Tasmanian culture facilities in plastic containers without water or sediment. All seawater used was drawn from exposed sections of coastline largely unaffected by freshwater runoff. However the quality of seawater for the University of Tasmania's (UTAS) bi-valve hatchery at Launceston is periodically affected by seaweed debris.

Broodstock Conditioning (Broodstock Trial 1)

Two separate groups of 200 broodstock clams were collected from Ansons Bay in August 1994 (Broodstock Trial 1) and transported to Marine Shellfish Hatcheries, Bicheno (Fig. 1). Gonad condition was visually assessed as intermediate (++) (Garland et al. 1993). Clams were scrubbed and rinsed with fresh water and placed in an indoor conditioning system consisting of a 1,100 L holding trough, recirculating pump, and header tank. Clams were held in plastic mesh baskets lined with 400 μm screen and filled with 2 to 4 mm quartz gravel. Water temperature was maintained at 13°C, and clams were fed a mixture of *Pavlova lutheri* (Droop) and Tahitian *Isochrysis* sp., supplemented with *Chaetoceros muelleri* (Lemmermann) at approximately 8 to 12×10^{10} cells/day. After approximately 8 weeks conditioning, clams were assessed as ripe (+++) (Garland et al. 1993), and 50 animals from each group were successfully spawned by cycling water temperatures between 13°C and 20°C.



Figure 1. Collection and larval rearing sites within Tasmania, Australia for the clam *Katelysia scalarina*.

Broodstock Maintenance (Broodstock Trial 2)

In December 1996 approximately 200 clams were collected from Ansons Bay. Twenty clams were fixed in 10% phosphate buffered formalin for later histology, and the remainder were reproductively conditioned in an indoor recirculating seawater system. The system at UTAS Launceston consisted of a 300-L reservoir and 4 × 50 L plastic bins (45 animals per bin) each with a floor area of 0.19 m². Beach sand (6-cm deep) was placed on the floor of two bins, so that clams could burrow, while clams in the other two bins were maintained without substrate. Water temperature was maintained at 20°C ± 0.5°C, and salinity remained constant at 34.5 ± 0.2 ppt. Clams were fed a daily ration of *P. lutheri*, Tahitian *Isochrysis* sp., and *Tetraselmis suecica* (Butcher) so that all feed was cleared within 24 h (Toba et al. 1992).

In January 1997 (6 weeks later), another sample of 20 clams was collected from the same site at Ansons Bay and preserved. A sample of 10 clams from each bin was also taken and preserved as above for later histology. A further 72 clams from the substrate bins were held out of water overnight and placed in 1 µm (nominal) filtered seawater (FSW) at 19°C the following morning, 45 minutes after which spawning commenced. Animals were allowed to spawn out in the tray yielding a total of 24 × 10⁶ eggs, with a

fertilization rate of 99%. These subsequently developed into active, healthy D larvae within 48 h.

Standard 4-µm paraffin sections were prepared from a 3-mm slice of the preserved samples, using the anterior edge of the foot as a reference (Howard and Smith 1983). These were stained using Mayer's hematoxylin and eosin Y. Gonads were staged for gametogenic development, using a modified staging system based on Dinamani (1974).

Spawning Induction

In summer 1995, clams from three sites (Georges Bay, Ansons Bay, and Swanwick) were collected for spawning trials 1 to 3 at UTAS, Launceston. Gonad condition by visual observation was assessed as moderate (++) (Garland et al. 1993). Clams failing to spawn after temperature cycling (20°C–26°C, at 30 min intervals), were injected via the anterior adductor muscle with various concentrations and volumes of serotonin (5-HT) (Table 1) (Gibbons and Castagna 1984, Gibbons and Castagna 1985, Heasman et al. 1994, O'Connor and Heasman 1995) and placed in 1 µm FSW at 25°C.

In spawning trial 4, strip spawnings were also attempted with moderate-to-ripe stock (++/+++) (Garland et al. 1993) that failed to spawn with temperature fluctuations. Ten females and six males were strip spawned by lacerating the gonad with sterile scalpel blades and flushing eggs and sperm into separate 1-L beakers with 1-µm FSW. Eggs were collected and screened through a 63-µm screen and resuspended in 1 L fresh FSW. Eggs were then treated with 0.25 mL of 2 M NH₄OH (0.5 × 10⁻³ M final concentration) for 45 minutes to aid breakdown of vesicle after Wada (1953) and Minaur (1969). Following this, eggs were resuspended in fresh FSW, and a dilute sperm solution was added. Fertilized eggs were placed in a 300-L larval rearing tank (LRT) at 20°C with low aeration.

Larval Rearing

Larval trials 1 to 4 (Table 2) were conducted in summer 1993 to 1994 at Shellfish Culture Pty. Ltd., a commercial bivalve hatchery at Bicheno. Broodstock were collected from various estuaries (Fig. 1), including Great Musselroe Bay (north coast), Swanwick on the east coast, and Cockle Creek in the south of the state.

Water used in spawning and larval rearing was 1-µm FSW (spawning and day 0) and 10-µm FSW thereafter. Disodium ethylenediaminetetra-acetic acid (EDTA) was added to day 0 water at 1 mg/L as per Utting and Helm (1985). With the exception of

TABLE 1.

Numbers of male and female *Katelysia scalarina* induced to spawn by injection of serotonin at different volumes and concentrations.

Spawning Trial	Number of Broodstock ^a	Collection Site	Injection Volume (µL)	Concentration ^b (mm)	Number Spawned	
					Male	Female
1	10	Georges Bay	100	10	1	0
1	10	Georges Bay	30	10	2	0
1	10	Ansons Bay	30	10	2	0
1	10	Swanwick	30	10	2	0
2	40	Ansons Bay	30	2	9	0
3	39	Ansons Bay	40	15	6	0

^a Sexes could not be visually differentiated prior to spawning; hence, number of broodstock = Σ(male + female).

^b Range of total amount of serotonin injected was (6 × 10⁻⁸ to 1 × 10⁻⁶ mole).

TABLE 2.

Mean fecundity and numbers of male and female *Katelysia scalarina* induced to spawn by thermal fluctuations.

Larval Trial	Number of Broodstock	Thermal Stimulus (°C)	U.V. Light	Number Spawned		Number of Eggs ($\times 10^6$)	Mean Fecundity (Eggs $\times 10^6$ /Clam)
				Male	Female		
1	No data ^a	20	No	No data ^a	No data ^a	4.5	No data ^a
2	186	24–27	Yes	50	62	150	2.4
3	98	20–26	Yes	40	29	20	0.7
4	230	20–26	Yes	22	26	26	1.0

^a Fertilized eggs collected following inadvertent overnight spawning of captive broodstock.

trial 1, ripe broodstock were held out of water for 12 h (Castagna and Manzi 1989) before being placed in a dark flat fiberglass trough (100 \times 600 \times 900 mm) filled with recirculating filtered seawater. Clams were encouraged to spawn by variously elevating water temperature by 3 to 6°C, as indicated in Table 2 (Loosanoff and Davis 1963). A UV light was included in the recirculating system to help maintain low bacterial levels and stimulate spawning (Bourne et al. 1989). Numbers of broodstock and spawning details are outlined in Table 2.

Larvae for trial 1 were obtained after broodstock spawned overnight in their holding tank. Eggs were collected the following morning and were rinsed through a 53- μ m screen and collected on 25- μ m mesh. Larval rearing then proceeded as for other trials. Larvae were cultured in 3,000-L and 15,000-L flat-bottom fiberglass tanks. For each trial, eggs were stocked at 7 to 10 eggs/mL, and larval densities were between 3 to 4 larvae/mL. Water temperatures were maintained at 24°C \pm 0.5°C. Tanks were cleaned and water was changed every 48 h after larvae had been retained on appropriate-sized nylon mesh screens. Larvae were fed a 1:1 (by cell number) mixture of *P. lutheri* and Tahitian *Isochrysis* sp. at approximately 10,000 cells/larvae/day (Toba et al. 1992).

For larval trial 5 in February 1996, ripe broodstock from Ansons Bay were encouraged to spawn at UTAS, Launceston via thermal fluctuations. Males and females were left to spawn together in the spawning tray. Fertilized eggs were screened through a 75- μ m screen to remove feces and other debris and retained on 25- μ m screen. Eggs were resuspended in fresh FSW and stocked in 300-L tanks at 25 eggs/mL. Equal quantities (by cell number) of *P. lutheri*, Tahitian *Isochrysis*, and *Chaetoceros calcitrans* (Paulsen) were fed at approximately 5,000 cells/larvae/day. Water temperatures were maintained at 20.5°C \pm 0.2°C. Tanks were cleaned and, water was changed every 48 h with 1- μ m UV sterilized FSW. On day 8, larvae were transferred to a 1,000-L fiberglass flat-bottom tank, and stocking densities were reduced to 1.2 larvae/mL. At day 18, 20,000 larvae were placed in a 200-mm diameter plastic downweller pot (with 132- μ m mesh) and treated with 10⁻⁴ M solution of the catecholamine norepinephrine for 60 min (Coon et al. 1986) to promote metamorphosis. Water was circulated within a 40-L bin by means of an air lift pump. Spat and juvenile clams were maintained for a further 48 days in the above system, with cleaning, feeding, and water changed every 48 hours.

RESULTS

K. scalarina can be reproductively conditioned in a relatively simple recirculating seawater system, and ripe broodstock can be maintained for more than a month for subsequent spawnings. His-

tological examination of clams collected from the wild for broodstock trial 2 in December 1996, revealed gonads to be ripe, with large numbers of mature ova or sperm in the gonad follicles. Subsequent retention of these animals for 6 weeks in a conditioning system did little to improve the extent of gonad development. However, examination of gonad tissue from wild stock in January 1997, revealed that at least a partial spawning had occurred, which was not mirrored by animals held in the broodstock system (Fig. 2).

In spawning trials 1 to 3, intramuscular injection of 5-HT encouraged some moderately conditioned males to spawn, releasing sperm with normal activity. However, in all cases, females failed to spawn (Table 1), despite variations in concentration and volume of 5-HT administered. Controls that were not injected did not spawn.

Stripped females (35–45 mm shell length) in spawning trial 4 yielded a total of 8 \times 10⁶ eggs, with a mean diameter of 70 μ m \pm

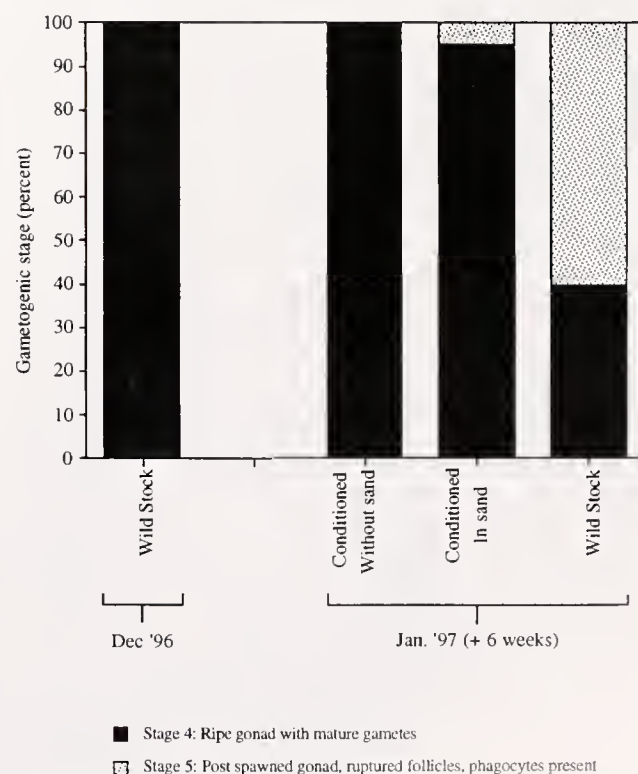


Figure 2. Gonad development in wild and captive *Katelysia scalarina* held for 6 weeks in a recirculating conditioning system, with and without substrate; n = 10.

2.5 μm . Fecundity of individuals ranged from 0.3×10^6 to 2.2×10^6 . Observed eggs were pear-shaped, with a prominent germinal vesicle. Survival of larvae from eggs treated with NH_4OH , was very low, falling to less than 1% by day 2 postfertilization. Moreover, many of the larvae that remained alive were abnormal and exhibited extensive fouling of the velum.

In larval rearing trials 2 to 5, ripe *K. scalarina* spawned readily in response to thermal stimulations of 3 to 6°C above ambient. Mean egg diameter was $69 \mu\text{m} \pm 2 \mu\text{m}$, and average fecundity ranged between 0.7×10^6 and 2.4×10^6 eggs/female (Table 2). Controls, not subjected to thermal stimulation, did not spawn.

Larval rearing was not always successful (Fig. 3). However, in all trials, development of fertilized eggs to trochophore stage occurred within 24 h and to D veligers, with a mean shell length of $110 \mu\text{m} \pm 1.3 \mu\text{m}$, by 48 h. Survival of larvae to day 5 was between 30 to 50%, but in all cases, was less than 1% by settlement. Growth of *K. scalarina* was rapid at 24°C, with larvae reaching a mean shell length of 200 μm by day 8 (Fig. 3a). Pediveligers of approximately 200 μm shell length, were first observed between day 9 and day 15, depending upon rearing temperatures, which were 24°C and 20.5°C, respectively (Fig. 3b, 4), and metamorphosis to spat was first observed by day 20 at 20.5°C (Fig. 4). Following metamorphosis, growth was exponential until day 53, after which it slowed abruptly, possibly in response to reduced water flows caused by fouling of the down-weller screen.

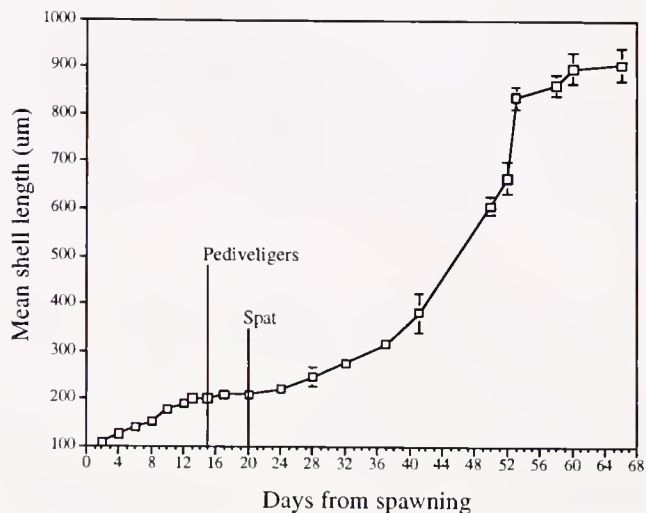


Figure 4. Growth (expressed as shell length) of *Katelaysia scalarina* reared at UTAS, Launceston (trial 5) during February to April 1996; points are mean \pm SE, (n = 5–20).

DISCUSSION

Broodstock

Broodstock conditioning systems are advantageous for maturing bivalves both within (Castagna and Kraeuter 1981, Castagna

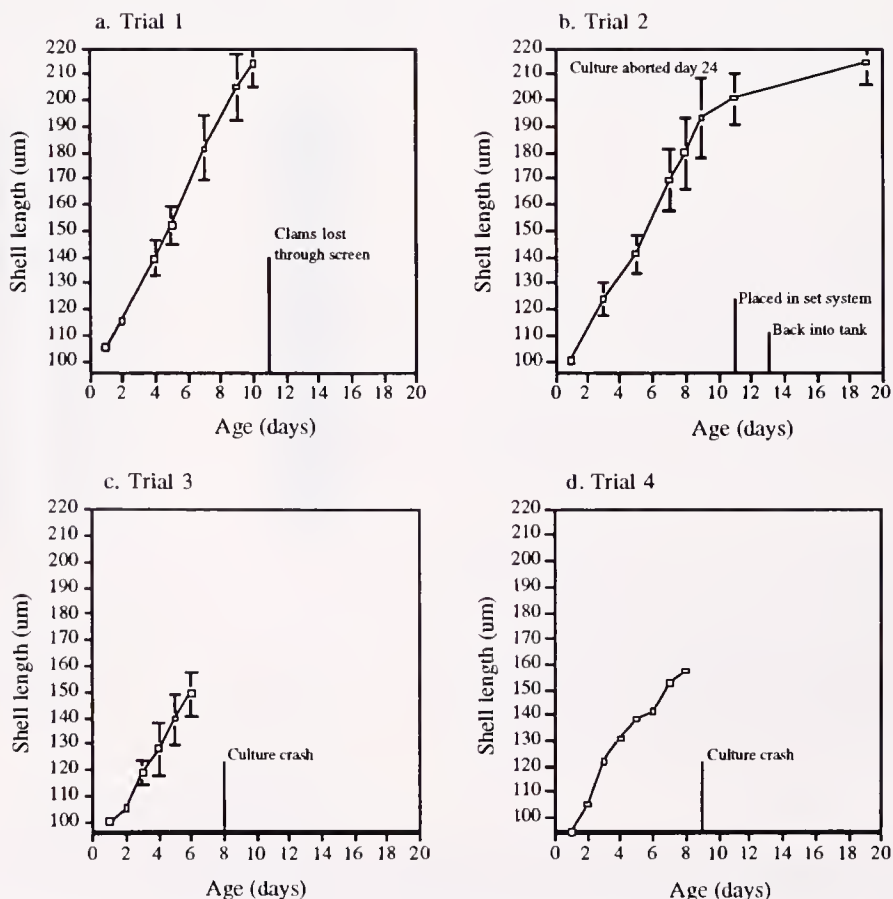


Figure 3. Results of four larval rearing trials of *Katelaysia scalarina*, conducted at Bicheno during summer 1993. Points are mean \pm SD, n = 20–38; a. and b. indicate rapid development (trials 1 and 2); c. and d. indicate characteristic larval crashes around days 6 to 8 (trials 3–4).

and Manzi 1989, Toba et al. 1992), and outside of the normal spawning season (Numaguchi 1997). *K. scalarina* broodstock held in clean sediment, could be conditioned at a relatively low temperature (13°C) (broodstock trial 1). Also, mature clams collected from the wild could be maintained in ripe condition within a recirculating system, despite indication of spawning of stock in the fishery during the experimental period (broodstock trial 2), (Fig. 2).

Spawning

Fecundity of *K. scalarina* was comparable to that for *K. rhytiphora* (Nell et al. 1994) and the Manila clam *Ruditapes philippinarum* Adams and Reeve (Utting et al. 1996, Laing and Lopez-Alvarado 1994), but less than that of the hard clam *Mercuraria mercenaria* (Castagna and Kraeuter 1981).

During spawning trials, both isolation of spawning male and female stock and mass spawnings in the tray were undertaken. We detected no deleterious effects from the latter, although more than 50 sperm per egg were observed on some occasions. Indeed, larvae displaying the greatest degree of activity were produced using this method. Nevertheless, *K. scalarina* has not always responded well to spawning induction protocols. On occasions, spawning has occurred up to 2 days after thermal stimulation, and neither serotonin injections nor strip spawnings have provided appropriate results. These difficulties may have arisen largely from variability in the quality of broodstock obtained directly from the wild. Spawning in two additional trials in the summer of 1996 to 1997 was relatively predictable (within 60 min of initiation of thermal stimulation) when broodstock in excellent condition were available.

Gibbons and Castagna (1985) found that injections of between 80 to 800 μmol of 5-HT into the adductor muscle of gravid *M. mercenaria* encouraged spawning; however, males were more responsive than females, and both concentration and absolute dose significantly affected spawning success. Numaguchi (1997) induced spawning in the common oriental clam *Meretrix lusoria* with a 125- μmol intragonadal injection of 5-HT, and other workers have induced spawning in bivalves with 10^{-3} M external applications (Ram et al. 1992). During our trials, the application of various concentrations of 5-HT failed to induce spawning in female broodstock, although some males did spawn. This may have been as a result of inadequate broodstock condition, although Ram and Nichols (1993) reported that injection of serotonin into zebra mussels induced ripe males to spawn but not females.

Strip spawnings using moderate-to-ripe broodstock were not successful, and larval mortality was very high (99%). In addition, many of the remaining larvae were deformed. In the rearing of *Pinctada maxima*, using stripped eggs treated with ammonium hydroxide, Minaur (1969) also reported up to 95% mortality of larvae to pediveliger stage, with some larvae showing deformed valves or other gross abnormalities. At this stage, it is unclear whether the deficiencies of this technique are primarily attributable to the method itself, such as possible toxic side effects of the NH_4OH used to promote breakdown of the germinal vesicle, or inadequately matured gametes. Stripped gametes from both this study and Minaur (1969) were primarily oocyte 3 as defined by Dinamani (1974). These oocytes are smaller and more irregular in shape as compared to free oocytes at final maturity (Dinamani 1974), and, as such, may have contributed to poor larval survival.

Larval Rearing

Our results suggest that *K. scalarina* cannot be reared reliably in hatcheries by simply adopting existing oyster hatchery tech-

niques. However, at 20°C, in trial 5, larval growth was comparable to *K. rhytiphora* (Nell et al. 1994) and similar to that for the Manila clam *R. philippinarum* (Utting and Spencer 1991).

In a number of the early trials run at 24°C, mass mortality occurred between days 8 and 9 (Fig. 3c,d). Larval crashes (between days 5 and 8) of commercial cultures of *K. scalarina* run at 16 to 18°C have also been reported (R. Pugh, pers. comm.). In both instances a herpes-like, viral pathogen has been found in larvae from some of the cultures investigated (J. Handler pers. comm.). Other failed cultures have been associated with bacterial infestations, a common problem with rearing of bivalve larvae (Gibbons and Blogoslawski 1989). Optimal temperatures for each successive stage in the early life history of *K. scalarina* must be identified. This should facilitate improved reliability of hatchery rearing as has been the case for such other bivalves as the commercial scallop *Pecten fumatus* (Reeve) (Heasman et al. 1996).

Results from larval trial 2 (Fig. 3b), suggest that further investigation of setting behavior should also be undertaken. Unlike *Crassostrea gigas* larvae, which may settle over a 24 to 48 h period (Beiras and Widdows 1995), our observations of *K. scalarina* seem similar to data for Manila clams *R. philippinarum*, which may indicate an extended settlement period (Utting and Spencer 1991). Transferring *K. scalarina* pediveligers to downweller oyster set systems containing a layer of ground scallop shell cultch for extended periods (as adopted in trial 2) proved to be unsatisfactory for this species, because larvae were eventually challenged by ciliates. Similarly, larvae from broodstock trial 1 developed well but did not metamorphose.

Although norepinephrine hastened permanent settlement (trial 5, Fig. 4), larval survival was low. Many larvae were invaded by a marine ciliate (resembling *Uronema nigricans*, see Munday et al. 1997) probably in response to increased bacterial numbers (Plunket and Hidu 1978). Because the effect of epinephrine or norepinephrine can vary with different species of oyster (Coon et al. 1986), specific work to determine appropriate concentration and duration for *K. scalarina* should be undertaken. In addition, larvae must be competent to metamorphose before inducement with epinephrine (Coon et al. 1986). With low larval numbers, this proved difficult to achieve for all larvae, because animals need to be graded into tight size groups before induction.

CONCLUSION

Based on some of the criteria outlined in Kraeuter and Castagna (1989), *Katelysia scalarina* have the potential of being an economically viable culture species. However, although broodstock can be adequately conditioned and subsequently spawned, larval rearing, and survival through metamorphosis, are still unreliable. The latter has primarily been because of the extended settlement period observed for this species, and the former because of the presence of a herpes-like viral pathogen. Further investigation of alternate types and dosing regimes for catecholamines as a means of enhancing settlement response is needed. Additional research is also required into the prevention and epidemiology of the herpes-like virus encountered during the course of this study. It is also recommended that the optimal rearing temperatures for the early life stages of this clam be identified.

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ESTIMATING TIME TO CRITICAL LEVELS OF *PERKINSUS MARINUS* IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*

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ABSTRACT A Visual-Basic program was developed as part of an Excel spreadsheet to estimate the time to a critical level (t_{crit}) of *Perkinsus marinus*, in eastern oysters *Crassostrea virginica*. The estimate is accomplished by assuming that a weighted incidence (WI) of disease of 1.5 is critical, converting measured WI values and the critical WI to parasite number, calculating a rate of change (r) of the parasite population using measured values of water temperature (T) and salinity (S), and solving for t_{crit} by simulation. The model produces estimates of t_{crit} and r using a long-term data set of T , S , and WI from the Terrebonne estuary of Louisiana. The model does not predict future values of WI since it cannot predict future trends in T and S ; however, regularly determining T and S , considering their interaction in a model, measuring WI at reasonable intervals, and iteratively estimating t_{crit} , should be useful to oyster management. Estimates of t_{crit} would support decisions concerning transplanting infected oysters to lower salinity areas, harvesting heavily infected populations early, and diverting freshwater into high-salinity estuaries.

KEY WORDS: Oyster, *Crassostrea virginica*, *Perkinsus marinus*, epizootiology, management, mathematical model, estuary

INTRODUCTION

Throughout its range, from Mexico (Burreson et al. 1994) to Maine (Ford 1996), *Perkinsus marinus* (= *Dermocystidium marinum*, Mackin et al. 1950) is an important cause of mortality (Ray and Mackin 1955, Andrews 1965) in eastern oysters, *Crassostrea virginica*. The success of oyster farming, therefore, often depends on the ability to manage oyster populations in estuaries where the parasite is widespread and/or abundant (Andrews and Ray 1988, Krantz and Jordan 1996). Management techniques of relevance to this study include transplanting infected oysters to lower salinity areas, early harvest of heavily infected populations, and freshwater diversion into high-salinity estuaries (Andrews and Ray 1988). These practices would clearly benefit by an estimate of the time to critical levels of infection. This estimate might be achieved by measuring water temperature and salinity, determining level of infection, calculating a rate of change of the parasite population, establishing a critical level of parasitism, and solving for the time to reach that critical level. The critical level of parasitism is set at a weighted incidence (WI) of 1.5, since a population of oysters with a WI of 2.0 contains an intense epidemic (Mackin 1962).

The purposes of this paper are to (1) present a long-term data set of temperature, salinity, and level of infection; (2) apply equations from an existing model (Hofmann et al. 1995, Powell et al. 1996) to a personal computer/spreadsheet format; (3) calculate time to critical level of infection using the data presented; and (4) evaluate the utility of the approach.

MATERIALS AND METHODS

Environmental parameters (temperature and salinity) and oysters were sampled from February 1992 to November 1997 at a site in the Terrebonne Basin of south central Louisiana. The reef was chosen to provide an accessible, unharvested, unplanted, subtidal, and persistent population of oysters. The site is at the junction of Bay Tambour and Bayou Petit Caillou, a local source of fresh water (Global Positioning System coordinates: 29°11'11.5"N, 90°39'56.0"W). Water depth varied from 0.3 to 0.6 m. Environmental parameters were measured weekly, whereas oysters were

sampled monthly. (Samples were not taken during the weeks of Hurricanes Andrew and Danny.) Water temperature (T) was measured to the nearest 0.1°C with a mercury thermometer, whereas salinity (S) was determined (nearest 0.5 ppt) using a refractometer (Behrens 1965).

Oysters were tonged at midmonth. Ten commercial-sized (75–124 mm) oysters were selected; a small piece of mantle tissue (about 4 mm²) was removed from each oyster and assayed for *P. marinus* after incubation in fluid thioglycollate for 1 week (Ray 1966). The intensity of infection was scored using Mackin's (1962) 0-to-5 scale as modified by Craig et al. (1989). Weighted incidence (WI) was calculated as the sum of the disease code numbers divided by the number of oysters in the sample, and percent infection (PI) of the sample was recorded.

A specific rate of parasite division (r_d) was related to T and S according to the formula of Hofmann et al. (1995):

$$r_d[T] = r_d[T_0] e^{0.0693(T(t)-T_0)} \quad (1)$$

where $e = 2.718$, $T_0 = 20^\circ\text{C}$, $r_d[T]$ = the specific rate of cell division (day⁻¹), $r_d[T_0]$ is the rate at 20°C and the exponent corresponds to a Q_{10} of 2.0. (At $T_0 = 20^\circ\text{C}$ and $S = 20$ ppt, $r_d[T_0] = 0.555$ day⁻¹; that is, the population doubles in size in about 2 days.) At 10 ppt or less, the rate of cell division is decreased and the equation takes the form:

$$r_d[T,S] = r_d[T_0,S_0] (S/10) e^{0.0693(T(t)-T_0)} \quad (2)$$

where $S_0 = 20$ ppt and $r_d[T_0,S_0] = r_d[T_0] = 0.555$ day⁻¹ (Hofmann et al. 1995). When parasite density is high, the specific rate of cell division decreases according to the equation:

$$r_d(\rho)_{j,k} = \beta r_d(T,S) (C_k/W_j)^\gamma \quad (3)$$

where $r_d(T,S)$ is determined from equation (2), j is oyster size, and k is infection class such that j,k is the infection level at a given size; β is a parasite scaling factor equal to 2.454×10^8 g ash-free dry weight (AFDW) cell⁻¹, and γ is a unit-less parasite density scaling factor equal to -1.5 according to Hofmann et al. (1995). The parasite number (C_k) was calculated from a measured WI value (x) according to the conversion of Choi et al. (1989):

$$C_k = 1409.9 \nu W_j (10^{0.64296\nu}). \quad (4)$$

The oyster weight (W_j) is 3.0 g AFDW, appropriate for oysters from 75 to 124 mm in length, and ν is 5.0, which converts oyster AFDW to wet weight (Hofmann et al. 1995).

A specific rate of parasite mortality (r_m) was likewise calculated according to Hofmann et al. (1995) as:

$$r_m(T, S) = r_{m0} e^{-\delta(S(t)/S_{oe})} e^{-\delta(T_o - 10/S_o)(S(t) - S_o)} \quad (5)$$

where $\delta = 0.081530^\circ\text{C}^{-1}$. Density effects on mortality rate follow the same relationship as was used for the density effect (equation 3) on parasite division rate:

$$r_m(\rho)_{j,k} = \beta r_m(T, S) (C_k/W_j)^\gamma. \quad (6)$$

The specific rate of change (r) was thus calculated as the appropriate r_d (equation 2 or 3) minus the appropriate r_m (equation 5 or 6).

A time to a critical level of parasitism is calculated by assuming that at a WI of about 1.5 an oyster population is experiencing mortalities. Mackin (1962) states: "a population of live oysters with a weighted incidence of 2.0 contains an intense epidemic, and more than half the population may be in advanced stages of the disease, with all of the individuals infected." A WI of 1.5 corresponds to a C_k of 194,500 hynospores per oyster of 3.0 g AFDW (equation 4).

The measured WI is converted to C_k according to equation 4, r is calculated as explained above using measured values for T and S , and the time to reach a critical level of parasitism (t_{crit}) is found by simulation. A Visual-Basic program, used to calculate r and as part of an Excel spreadsheet module, is shown in Table 1.

RESULTS

Table 2 provides weekly measured environmental data (T and S), midmonthly measured levels of infection (WI and PI), and conversions and calculations generated by the model (C_k , t_{crit} , r_d , r_m , and r). Water temperature varied from 6.5°C (12/19/96) to 31.9°C (7/30/93), whereas S ranged from 1.0 ppt (12/23/93 and 12/30/93) to 25.0 ppt (5/18/95). (Mid-month T and S are plotted in Fig. 1a and 1b, respectively.) Weighted incidence was highest (2.66) on 11/14/96 and lowest (0.00) on 3/19/92 (Fig. 1c); PI ranged from 0 to 100%. Values of C_k , calculated from measured WI values, ranged from 21,148 (even when WI was 0.00) to 1,085,308 (at WI = 2.66). (That $C_k = 21,148$ when WI = 0.00 is implied in equation 4 and is due to false negatives of the thioglycollate method.) Greatest values of parasite division rate (r_d) were typically found in the late summer and early fall (as high as 0.582 on 8/17/95). Likewise, greatest values of parasite mortality rate (r_m) were commonly found during the winter and early spring (as high as 0.553 on 4/7/94 and 12/19/96). The specific rate of change (r), the difference between r_d and r_m , is thus generally greatest in the late summer and early fall (Fig. 1d). The t_{crit} is, of course, lowest when r is highest (Fig. 1e).

The behavior of the model was observed (Fig. 2) by calculating t_{crit} at various combinations of T (5, 10, 15, 20, 25, 30, and 35°C) and S (5, 10, 15, 20, 25, 30 and 35 ppt) using initial C_k values of 10,000 (Fig. 2a), 50,000 (Fig. 2b), 100,000 (Fig. 2c), and 150,000 hynospores (Fig. 2d). (To make the graphs easier to read, values of $t_{crit} \geq 60$ were assigned a value of 60 days; this is also a practical upper limit, since with monthly sampling, values ≥ 60 days should not be of critical concern.) As T , S , and initial C_k

increase, t_{crit} progressively decreases. For example, consider conditions representing a cool, wet winter (10°C, 10 ppt) versus a hot, dry summer (30°C, 25 ppt) at different initial values of C_k (Fig. 2). With an initial C_k of 10,000 hynospores (Fig. 2a) at 10°C and 10 ppt, t_{crit} is ≥ 60 days; at 30°C and 25 ppt, t_{crit} is < 10 days. With an initial C_k of 150,000 hynospores (Fig. 2d) at 10°C and 10 ppt, t_{crit} is again ≥ 60 days, whereas at 30°C and 25 ppt, t_{crit} is < 1 day.

DISCUSSION

The relationship of temperature and salinity to levels of infection of *P. marinus* in the eastern oyster is well documented (e.g., see Mackin et al. 1950, Ray and Mackin 1955, Mackin 1962, Soniat 1985). These two factors, however important, do not explain most of the variation in levels of infection observed in the field. Soniat (1985), for example, showed that the interaction of temperature and salinity explained only about 49% of the variation in weighted incidence of infection. Clearly other factors are important. These include, but may not be limited to, pollution and other human influences, host nutrition and growth, spawning and reproduction, age and resistance, oyster density and distribution, and disease vectors (Soniat 1996)—factors that comprise part of the oysters' history (Powell et al. 1996). However, it is impractical, if not intractable, to ascertain and measure all of the factors that determine levels of *P. marinus* in oysters. Nevertheless, determining T and S , considering their interaction in a model, measuring WI at reasonable intervals, and iteratively calculating a time to a critical level of parasitism (t_{crit}) should be useful to oyster management.

The data to support the model, namely, environmental data (T and S) and WI of *P. marinus*, have considerably distinct attributes. The collection of environmental data is easily automated, relatively inexpensive, and in some cases instantaneous. In contrast, obtaining WI data is relatively expensive and time consuming, and not amenable to automation. Applications of the model should therefore make best use of limited WI data and abundant environmental data. This might best be achieved by a stratified sampling program in which oysters are sampled for *P. marinus* in a few broad salinity zones. Decisions could therefore be made on a zone-by-zone basis, as t_{crit} changes from season to season. The model justifies a broad delineation of zones in which the levels of parasitism could be characterized as (1) of "no immediate concern" (e.g., $t_{crit} > 30$ days), (2) of "current concern" (e.g., $t_{crit} < 30$ days), and (3) of "immediate concern" (t_{crit} approaching 0 days). Management decisions that would be aided by a better estimate of t_{crit} include early harvesting oysters in the "zone of immediate concern," transplanting of oysters to the "zones of no immediate concern" (Andrews and Ray 1988), and timing and positioning freshwater inputs into estuaries (Chatry et al. 1983). Sampling decisions would also benefit since when t_{crit} becomes less than the sample interval (in this case 1 mo), more fine-scaled measurements might be initiated.

The model behaves reasonably in that as T , S , and initial C_k increase, the t_{crit} progressively and predictably decreases (Fig. 2). Calculations of t_{crit} using the data set (Table 2) also show the expected decrease in the late summer and early fall (Ray and Mackin 1955, Mackin 1962). The model does not predict future values of WI because it cannot predict future values of T and S . It can be used to determine trends in t_{crit} and help reduce the risks associated with oyster cultivation. Thus, a single low value of t_{crit}

TABLE 1.

A Visual-Basic program used to calculate time to critical level of *P. marinus* (t_{crit}), using temperature (T), salinity (S), and parasite number (C_k) as input values.

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Const normal density = 500000
Const alpha = 0.06931
Const beta = 3.454 * (10 ^ 8)
Const delta = -0.08153
Const gamma = -1.5
Const S0 = 20
Const T0 = 20
Const crit = 194800

Function rd (T As Double, S As Double, Ck As Double, Wj As Double) As Double
Const Rd0 = 0.25

If S < 10 Then
    mult = S / 10
Else
    mult = 1
End If

rdt = Rd0 * mult * Exp (alpha * (T - T0))
If (Ck <= normal density) Then
    rd = rdt
Else
    rd = beta * rdt * [(Ck / Wj) ^ gamma]
End If

End Function

Function rm (T As Double, S As Double, Ck As Double, Wj As Double) As Double
Const rm0 = 0.3

If ((T - 10) >= 10) Then
    e = T - 10
Else
    e = 10
End If

rmt = rm0 * Exp (delta * (S / S0) * e) * Exp (delta * [(T0 - 10) / S0] * (S - S0))

If (Ck <= normal density) Then
    rm = rmt
Else
    rm = beta * rmt * [(Ck / Wj) ^ gamma]
End If

End Function

Function tCrit (T As Double, S As Double, Ck As Double, Wj As Double) As Double
Tm = 0
r = 0
While (Ck <= crit)
    Tm = Tm + 1
    rdt = rd (T, S, Ck, Wj)
    rmt = rm (T, S, Ck, Wj)
    r = rdt - rmt
    Ck = Ck * (1 + r)
    If Tm > 999 Then Ck = crit + 1
Wend
tCrit = Tm

End Function

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is not necessarily cause for alarm. For example, in 1992, 1993, and 1995, t_{crit} was often <7 days, but never reached 0 days the following week. In contrast, in 1994, 1996, and 1997, t_{crit} reached 0 days on numerous occasions (Table 2).

As constructed, the model errs on the side of caution for two

reasons. First, although a W1 of 1.5 in oysters is of concern it is not catastrophic but merely an indication of possible problems. Secondly, the model does not consider recruitment and growth, in which young uninfected oysters replace the old, infected, and dying ones. (The more complete and complex model of Hoffman et

TABLE 2.

Sample date, weekly temperature (T, °C) and salinity (S, ppt), weighted incidence (WI), and percent infection (PI) measured at mid-month. Values of initial parasite number (C_k) are converted from *mid-monthly* WI values for each *weekly* combination of T and S. (Thus, WI and PI are repeated in the table.) A calculated time to critical level of *P. marinus* (t_{crit} , days) is shown, with corresponding values of specific rate of parasite division (r_d , day⁻¹), specific rate of parasite mortality (r_m , day⁻¹), and the specific rate of change (r , day⁻¹), where $r = r_d - r_m$.

Date	T	S	WI	PI	C_k	t_{crit}	r_d	r_m	r
2/20/1992	16.4	2.0							
2/27/1992	14.7	2.0							
3/5/1992	20.1	15.0							
3/12/1992	15.5	12.0							
3/19/1992	21.0	14.0	0.00	0.00	21,149	37	0.268	0.205	0.063
3/26/1992	19.2	12.0	0.00	0.00	21,149	1000	0.237	0.255	-0.018
4/2/1992	16.0	7.0	0.00	0.00	21,149	1000	0.133	0.383	-0.251
4/9/1992	20.8	15.0	0.00	0.00	21,149	32	0.264	0.190	0.074
4/16/1992	24.1	19.0	0.23	40.00	29,728	10	0.332	0.105	0.227
4/23/1992	22.9	15.0	0.23	40.00	29,728	15	0.306	0.167	0.139
4/30/1992	19.4	11.0	0.23	40.00	29,728	1000	0.240	0.277	-0.037
5/7/1992	19.8	11.0	0.23	40.00	29,728	1000	0.247	0.277	-0.030
5/14/1992	26.4	20.0	0.67	50.00	57,025	5	0.390	0.079	0.311
5/21/1992	26.8	20.0	0.67	50.00	57,025	5	0.401	0.076	0.324
5/28/1992	25.9	18.0	0.67	50.00	57,025	6	0.376	0.101	0.275
6/4/1992	27.1	19.0	0.67	50.00	57,025	5	0.409	0.083	0.326
6/11/1992	29.0	22.0	0.67	50.00	57,025	4	0.466	0.050	0.416
6/18/1992	31.2	22.0	0.70	70.00	59,614	3	0.543	0.041	0.502
6/24/1992	30.2	16.0	0.70	70.00	59,614	4	0.507	0.095	0.412
7/2/1992	28.6	23.0	0.70	70.00	59,614	4	0.454	0.046	0.407
7/9/1992	31.2	20.0	0.70	70.00	59,614	3	0.543	0.053	0.490
7/16/1992	26.7	22.0	0.63	80.00	53,746	5	0.398	0.062	0.336
7/23/1992	30.0	16.0	0.63	80.00	53,746	4	0.500	0.096	0.404
7/30/1992	30.4	18.5	0.63	80.00	53,746	4	0.514	0.068	0.446
8/6/1992	31.0	15.5	0.63	80.00	53,746	4	0.536	0.096	0.440
8/12/1992	29.9	18.0	0.87	70.00	76,675	3	0.497	0.076	0.421
8/20/1992	28.0	10.5	0.87	70.00	76,675	5	0.435	0.205	0.231
9/3/1992	29.6	14.0	0.87	70.00	76,675	4	0.486	0.125	0.361
9/10/1992	30.0	11.5	0.87	70.00	76,675	4	0.500	0.166	0.334
9/17/1992	25.6	15.0	0.73	70.00	62,322	6	0.369	0.142	0.227
9/24/1992	26.0	11.5	0.73	70.00	62,322	7	0.379	0.200	0.179
10/1/1992	20.8	15.0	0.73	70.00	62,322	16	0.264	0.190	0.074
10/8/1992	23.0	19.5	0.73	70.00	62,322	7	0.308	0.109	0.199
10/15/1992	24.0	17.5	0.80	90.00	69,127	6	0.330	0.122	0.208
10/22/1992	22.2	22.0	0.80	90.00	69,127	6	0.291	0.093	0.199
10/29/1992	25.0	21.5	0.80	90.00	69,127	5	0.354	0.076	0.278
11/5/1992	14.8	12.5	0.80	90.00	69,127	1000	0.174	0.245	-0.070
11/12/1992	20.0	16.0	1.30	100.00	144,920	5	0.250	0.184	0.066
11/19/1992	18.2	19.5	1.30	100.00	144,920	4	0.221	0.138	0.082
11/25/1992	17.6	14.0	1.30	100.00	144,920	1000	0.212	0.217	-0.005
12/3/1992	12.4	12.0	1.30	100.00	144,920	1000	0.148	0.255	-0.107
12/10/1992	13.8	11.0	1.30	100.00	144,920	1000	0.163	0.277	-0.114
12/17/1992	15.4	7.5	0.53	90.00	46,350	1000	0.136	0.368	-0.232
12/23/1992	20.8	7.0	0.53	90.00	46,350	1000	0.185	0.374	-0.190
12/30/1992	18.0	9.0	0.53	90.00	46,350	1000	0.196	0.325	-0.130
1/6/1993	11.4	9.5	0.53	90.00	46,350	1000	0.131	0.312	-0.182
1/14/1993	13.4	2.5	0.47	70.00	42,410	1000	0.040	0.553	-0.513
1/21/1993	18.8	7.0	0.47	70.00	42,410	1000	0.161	0.383	-0.222
1/28/1993	12.4	7.0	0.47	70.00	42,410	1000	0.103	0.383	-0.280
2/4/1993	14.4	8.5	0.47	70.00	42,410	1000	0.144	0.339	-0.195
2/11/1993	15.8	14.5	0.47	70.00	42,410	1000	0.187	0.208	-0.021
2/18/1993	13.0	3.0	0.07	20.00	23,458	1000	0.046	0.531	-0.485
2/25/1993	16.0	17.0	0.07	20.00	23,458	108	0.189	0.170	0.020
3/4/1993	15.0	8.0	0.07	20.00	23,458	1000	0.141	0.353	-0.212
3/11/1993	19.0	14.0	0.07	20.00	23,458	128	0.233	0.217	0.017
3/18/1993	13.5	8.5	0.27	60.00	31,541	1000	0.135	0.339	-0.204

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TABLE 2.

continued

Date	T	S	W1	PI	Ck	t _{Crit}	rd	rm	r
3/25/1993	19.7	15.5	0.27	60.00	31,541	36	0.245	0.192	0.053
4/1/1993	20.5	10.5	0.27	60.00	31,541	1000	0.259	0.282	-0.023
4/9/1993	16.9	9.0	0.27	60.00	31,541	1000	0.181	0.325	-0.144
4/15/1993	20.9	11.5	0.93	100.00	83,798	74	0.266	0.254	0.012
4/22/1993	19.0	6.5	0.93	100.00	83,798	1000	0.152	0.399	-0.247
4/29/1993	22.2	15.5	0.93	100.00	83,798	8	0.291	0.167	0.124
5/6/1993	24.5	11.0	0.93	100.00	83,798	8	0.342	0.226	0.116
5/13/1993	24.7	5.0	0.50	70.00	44,336	1000	0.173	0.410	-0.237
5/20/1993	25.4	14.5	0.50	70.00	44,336	8	0.363	0.151	0.212
5/27/1993	25.2	11.0	0.50	70.00	44,336	12	0.358	0.219	0.139
6/4/1993	28.0	15.5	0.50	70.00	44,336	6	0.435	0.116	0.320
6/11/1993	30.4	14.0	0.50	70.00	44,336	5	0.514	0.120	0.394
6/17/1993	29.3	14.5	0.63	80.00	53,746	5	0.476	0.120	0.356
6/24/1993	27.8	14.5	0.63	80.00	53,746	5	0.429	0.131	0.298
7/2/1993	30.6	14.5	0.63	80.00	53,746	4	0.521	0.111	0.410
7/9/1993	29.7	9.0	0.63	80.00	53,746	7	0.441	0.228	0.213
7/16/1993	29.2	10.5	0.43	80.00	39,972	7	0.473	0.194	0.279
7/23/1993	29.0	5.5	0.43	80.00	39,972	1000	0.257	0.354	-0.097
7/30/1993	31.9	14.0	0.43	80.00	39,972	5	0.570	0.110	0.461
8/5/1993	31.5	13.5	0.43	80.00	39,972	5	0.555	0.120	0.435
8/12/1993	31.4	10.0	0.07	10.00	23,458	7	0.551	0.188	0.362
8/19/1993	31.0	5.5	0.07	10.00	23,458	1000	0.295	0.338	-0.044
8/26/1993	29.0	11.5	0.07	10.00	23,458	9	0.466	0.174	0.292
9/2/1993	29.9	10.0	0.07	10.00	23,458	9	0.497	0.200	0.296
9/9/1993	30.1	8.0	0.07	10.00	23,458	16	0.403	0.254	0.149
9/16/1993	27.7	7.0	0.37	70.00	36,574	1000	0.298	0.308	-0.009
9/23/1993	30.0	12.0	0.37	70.00	36,574	6	0.500	0.156	0.344
10/2/1993	24.0	12.0	0.37	70.00	36,574	15	0.330	0.210	0.120
10/7/1993	26.0	12.0	0.37	70.00	36,574	10	0.379	0.190	0.189
10/14/1993	23.0	12.5	0.77	80.00	66,124	12	0.308	0.210	0.098
10/21/1993	26.9	14.0	0.77	80.00	66,124	5	0.403	0.146	0.257
10/28/1993	20.9	9.0	0.77	80.00	66,124	1000	0.239	0.315	-0.075
11/4/1993	27.2	8.0	0.77	80.00	66,124	23	0.329	0.279	0.050
11/11/1993	15.2	11.0	0.77	80.00	66,124	1000	0.179	0.277	-0.097
11/18/1993	19.6	8.0	0.50	50.00	44,336	1000	0.195	0.353	-0.159
11/26/1993	17.6	9.0	0.50	50.00	44,336	1000	0.191	0.325	-0.135
12/2/1993	17.0	15.0	0.50	50.00	44,336	424	0.203	0.200	0.004
12/9/1993	17.2	11.0	0.50	50.00	44,336	1000	0.206	0.277	-0.071
12/16/93	11.8	7.0	0.27	30.00	31,541	1000	0.099	0.383	-0.284
12/23/1993	9.5	1.0	0.27	30.00	31,541	1000	0.012	0.625	-0.613
12/30/1993	9.9	1.0	0.27	30.00	31,541	1000	0.012	0.625	-0.612
1/6/1994	11.4	9.5	0.27	30.00	31,541	1000	0.131	0.312	-0.182
1/13/1994	12.2	9.5	0.13	20.00	25,637	1000	0.138	0.312	-0.174
1/20/1994	8.5	13.0	0.13	20.00	25,637	1000	0.113	0.235	-0.122
1/27/1994	17.8	15.5	0.13	20.00	25,637	89	0.215	0.192	0.023
2/3/1994	9.2	9.0	0.13	20.00	25,637	1000	0.106	0.325	-0.219
2/10/1994	21.6	19.5	0.13	20.00	25,637	14	0.279	0.122	0.158
2/16/1994	13.9	14.0	0.23	30.00	29,728	1000	0.164	0.217	-0.053
2/24/1994	14.9	8.0	0.23	30.00	29,728	1000	0.140	0.353	-0.213
3/3/1994	12.2	5.5	0.23	30.00	29,728	1000	0.080	0.433	-0.353
3/10/1994	13.3	4.0	0.23	30.00	29,728	1000	0.063	0.489	-0.426
3/17/1994	17.2	16.0	0.43	70.00	39,972	73	0.206	0.184	0.022
3/24/1994	23.7	14.0	0.43	70.00	39,972	12	0.323	0.175	0.148
3/31/1994	16.8	9.0	0.43	70.00	39,972	1000	0.180	0.325	-0.145
4/7/1994	16.0	2.5	0.43	70.00	39,972	1000	0.047	0.553	-0.506
4/14/1994	23.0	16.0	0.30	50.00	32,974	13	0.308	0.151	0.157
4/21/1994	22.4	7.0	0.30	50.00	32,974	1000	0.207	0.358	-0.151
4/28/1994	26.6	14.5	0.30	50.00	32,974	8	0.395	0.141	0.254
5/5/1994	23.2	5.5	0.30	50.00	32,974	1000	0.172	0.403	-0.231
5/12/1994	29.0	11.5	0.47	70.00	42,410	6	0.466	0.174	0.292
5/19/1994	25.0	5.0	0.47	70.00	42,410	1000	0.177	0.407	-0.231

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TABLE 2.

continued

Date	T	S	WI	PI	Ck	t _{Crit}	rd	rm	r
5/26/1994	26.9	13.5	0.47	70.00	42,410	7	0.403	0.154	0.249
6/3/1994	27.6	7.5	0.47	70.00	42,410	60	0.318	0.292	0.026
6/8/1994	29.8	10.5	0.47	70.00	42,410	6	0.493	0.189	0.304
6/17/1994	31.6	8.0	0.27	60.00	31,541	10	0.447	0.242	0.205
6/23/1994	28.0	15.0	0.27	60.00	31,541	7	0.435	0.122	0.313
6/29/1994	29.5	8.0	0.27	60.00	31,541	16	0.386	0.259	0.127
7/8/1994	27.9	14.5	0.27	60.00	31,541	7	0.432	0.130	0.302
7/15/1994	28.2	7.5	0.63	90.00	53,746	30	0.331	0.286	0.045
7/21/1994	29.2	12.0	0.63	90.00	53,746	5	0.473	0.162	0.311
7/28/1994	27.0	5.0	0.63	90.00	53,746	1000	0.203	0.391	-0.188
8/4/1994	28.4	13.0	0.63	90.00	53,746	5	0.447	0.151	0.297
8/11/1994	28.9	11.5	0.63	90.00	53,746	6	0.463	0.175	0.288
8/18/1994	29.2	16.5	0.50	70.00	44,336	5	0.473	0.095	0.378
8/25/1994	29.7	7.5	0.50	70.00	44,336	17	0.367	0.273	0.094
9/1/1994	29.7	16.5	0.50	70.00	44,336	5	0.490	0.092	0.398
9/8/1994	30.1	13.0	0.50	70.00	44,336	5	0.503	0.138	0.366
9/15/1994	26.5	16.0	1.47	80.00	186,394	1	0.392	0.120	0.272
9/22/1994	27.7	14.0	1.47	80.00	186,394	1	0.426	0.140	0.287
9/29/1994	26.0	19.0	1.47	80.00	186,394	1	0.379	0.090	0.288
10/6/1994	26.2	16.0	1.47	80.00	186,394	1	0.384	0.123	0.261
10/13/1994	20.9	15.5	1.50	100.00	194,859	0	0.266	0.181	0.085
10/18/1994	25.8	23.0	1.50	100.00	194,859	0	0.374	0.060	0.313
10/27/1994	20.4	13.0	1.50	100.00	194,859	0	0.257	0.230	0.027
11/3/1994	22.6	19.5	1.50	100.00	194,859	0	0.299	0.112	0.187
11/10/1994	24.8	18.5	1.50	100.00	194,859	0	0.349	0.104	0.244
11/17/1994	19.9	16.0	1.07	100.00	103,097	11	0.248	0.184	0.064
11/22/1994	20.2	18.0	1.07	100.00	103,097	7	0.253	0.154	0.100
12/1/1994	13.8	18.0	1.07	100.00	103,097	100	0.163	0.156	0.006
12/9/1994	20.2	11.5	1.07	100.00	103,097	1000	0.253	0.263	-0.009
12/15/1994	14.5	13.0	0.80	100.00	69,127	1000	0.171	0.235	-0.064
12/22/1994	15.7	17.5	0.80	100.00	69,127	46	0.186	0.163	0.023
12/29/1994	15.2	16.0	0.80	100.00	69,127	1000	0.179	0.184	-0.005
1/5/1995	17.3	6.0	0.80	100.00	69,127	1000	0.124	0.416	-0.291
1/12/1995	18.3	15.5	0.43	70.00	39,972	53	0.222	0.192	0.031
1/19/1995	13.2	9.5	0.43	70.00	39,972	1000	0.148	0.312	-0.164
1/26/1995	12.0	7.5	0.43	70.00	39,972	1000	0.108	0.368	-0.260
2/2/1995	15.8	9.5	0.43	70.00	39,972	1000	0.178	0.312	-0.135
2/9/1995	10.0	5.5	0.43	70.00	39,972	1000	0.069	0.433	-0.364
2/16/1995	17.2	13.5	0.23	50.00	29,728	1000	0.206	0.226	-0.020
2/23/1995	17.4	14.0	0.23	50.00	29,728	1000	0.209	0.217	-0.008
3/2/1995	14.0	4.5	0.23	50.00	29,728	1000	0.074	0.470	-0.396
3/9/1995	10.7	5.5	0.23	50.00	29,728	1000	0.072	0.433	-0.361
3/16/1995	21.0	20.5	0.43	80.00	39,972	12	0.268	0.117	0.151
3/23/1995	23.3	22.0	0.43	80.00	39,972	8	0.314	0.084	0.230
3/30/1995	17.4	18.0	0.43	80.00	39,972	31	0.209	0.156	0.053
4/6/1995	19.2	17.0	0.43	80.00	39,972	25	0.237	0.170	0.067
4/13/1995	22.0	13.0	0.77	90.00	66,124	15	0.287	0.211	0.076
4/20/1995	25.4	20.0	0.77	90.00	66,124	5	0.363	0.085	0.278
4/27/1995	21.5	16.5	0.77	90.00	66,124	10	0.277	0.160	0.118
5/4/1995	25.0	20.0	0.77	90.00	66,124	5	0.354	0.088	0.265
5/11/1995	27.0	17.0	0.77	90.00	66,124	5	0.406	0.104	0.302
5/18/1995	27.6	25.0	0.93	90.00	83,798	3	0.423	0.041	0.383
5/26/1995	28.4	20.5	0.93	90.00	83,798	3	0.447	0.063	0.384
6/2/1995	27.5	14.0	0.93	90.00	83,798	4	0.420	0.141	0.279
6/9/1995	29.2	15.5	0.93	90.00	83,798	3	0.473	0.107	0.366
6/16/1995	25.9	9.0	0.40	70.00	38,235	23	0.339	0.262	0.077
6/23/1995	29.4	14.0	0.40	70.00	38,235	6	0.480	0.127	0.353
6/30/1995	26.4	6.0	0.40	70.00	38,235	1000	0.234	0.355	-0.122
7/6/1995	29.2	13.0	0.40	70.00	38,235	6	0.473	0.144	0.329
7/13/1995	30.6	14.0	0.17	40.00	27,201	6	0.521	0.118	0.403

continued on next page

TABLE 2.

continued

Date	T	S	WI	PI	Ck	t _{Crit}	rd	rm	r
7/20/1995	31.0	10.0	0.17	40.00	27,201	7	0.536	0.192	0.344
7/27/1995	31.2	19.0	0.17	40.00	27,201	5	0.543	0.060	0.483
8/2/1995	28.0	16.0	0.17	40.00	27,201	7	0.435	0.109	0.326
8/10/1995	31.0	18.0	0.17	40.00	27,201	6	0.536	0.070	0.466
8/17/1995	32.2	14.0	0.23	40.00	29,728	5	0.582	0.108	0.474
8/24/1995	30.8	16.0	0.23	40.00	29,728	6	0.528	0.091	0.438
8/31/1995	30.6	15.5	0.23	40.00	29,728	6	0.521	0.098	0.423
9/7/1995	27.7	17.0	0.23	40.00	29,728	7	0.426	0.099	0.327
9/14/1995	30.4	15.5	1.23	100.00	130,653	2	0.514	0.099	0.415
9/21/1995	30.0	19.5	1.23	100.00	130,653	2	0.500	0.062	0.438
9/28/1995	27.0	19.0	1.23	100.00	130,653	2	0.406	0.084	0.322
10/5/1995	23.8	20.5	1.23	100.00	130,653	2	0.325	0.093	0.233
10/12/1995	24.5	19.0	1.27	90.00	138,624	2	0.342	0.102	0.240
10/19/1995	23.0	23.0	1.27	90.00	138,624	2	0.308	0.078	0.229
10/26/1995	23.0	23.0	1.27	90.00	138,624	2	0.308	0.078	0.229
11/2/1995	24.6	24.0	1.27	90.00	138,624	2	0.344	0.061	0.283
11/9/1995	16.4	19.0	1.27	90.00	138,624	7	0.195	0.144	0.051
11/16/1995	17.4	18.0	0.73	80.00	62,322	23	0.209	0.156	0.053
11/24/1995	18.0	18.0	0.73	80.00	62,322	20	0.218	0.156	0.061
11/30/1995	15.0	13.5	0.73	80.00	62,322	1000	0.177	0.226	-0.049
12/7/1995	22.2	16.0	0.73	80.00	62,322	10	0.291	0.159	0.132
12/12/1995	10.4	17.5	0.70	90.00	59,614	1000	0.129	0.163	-0.034
12/21/1995	9.0	12.5	0.70	90.00	59,614	1000	0.117	0.245	-0.128
12/29/1995	6.6	12.0	0.70	90.00	59,614	1000	0.099	0.255	-0.156
1/3/1996	9.4	11.5	0.70	90.00	59,614	1000	0.120	0.265	-0.146
1/11/1996	11.0	14.5	0.70	90.00	59,614	1000	0.134	0.208	-0.074
1/18/1996	17.6	20.0	1.27	100.00	138,624	5	0.212	0.133	0.079
1/25/1996	11.6	11.0	1.27	100.00	138,624	1000	0.140	0.277	-0.137
2/1/1996	13.4	12.0	1.27	100.00	138,624	1000	0.158	0.255	-0.097
2/8/1996	10.0	16.0	1.27	100.00	138,624	1000	0.125	0.184	-0.059
2/15/1996	15.9	15.5	0.33	50.00	34,471	1000	0.188	0.192	-0.003
2/22/1996	20.0	18.0	0.33	50.00	34,471	20	0.250	0.156	0.094
2/29/1996	15.7	4.0	0.33	50.00	34,471	1000	0.074	0.489	-0.415
3/7/1996	17.0	14.0	0.33	50.00	34,471	1000	0.203	0.217	-0.013
3/14/1996	19.0	22.0	1.27	80.00	138,624	3	0.233	0.113	0.120
3/21/1996	12.2	15.0	1.27	80.00	138,624	1000	0.146	0.200	-0.054
3/28/1996	16.9	18.5	1.27	80.00	138,624	7	0.202	0.150	0.052
4/4/1996	19.2	18.0	1.27	80.00	138,624	5	0.237	0.156	0.080
4/11/1996	19.0	21.5	1.27	80.00	138,624	4	0.233	0.117	0.116
4/18/1996	20.7	21.0	1.90	100.00	352,293	0	0.262	0.115	0.147
4/25/1996	20.6	15.5	1.90	100.00	352,293	0	0.261	0.184	0.076
5/2/1996	21.1	19.0	1.90	100.00	352,293	0	0.270	0.132	0.138
5/9/1996	26.7	21.0	1.90	100.00	352,293	0	0.398	0.069	0.329
5/16/1996	26.0	19.5	2.53	100.00	895,300	0	0.154	0.035	0.120
5/23/1996	28.6	19.5	2.53	100.00	895,300	0	0.185	0.028	0.157
5/29/1996	29.2	21.5	2.53	100.00	895,300	0	0.193	0.021	0.171
6/4/1996	29.0	15.5	2.53	100.00	895,300	0	0.190	0.044	0.146
6/14/1996	27.3	16.5	0.97	100.00	88,910	3	0.415	0.108	0.307
6/20/1996	29.7	15.0	0.97	100.00	88,910	3	0.490	0.110	0.379
6/28/1996	28.6	7.5	0.97	100.00	88,910	15	0.340	0.283	0.058
7/5/1996	30.6	8.5	0.97	100.00	88,910	5	0.443	0.235	0.208
7/12/1996	31.1	9.0	0.97	100.00	88,910	4	0.486	0.217	0.269
7/19/1996	30.0	15.5	0.33	70.00	34,471	6	0.500	0.102	0.398
7/25/1996	29.1	10.5	0.33	70.00	34,471	8	0.470	0.195	0.275
8/2/1996	28.5	9.5	0.33	70.00	34,471	10	0.428	0.225	0.203
8/9/1996	30.0	9.5	0.33	70.00	34,471	8	0.475	0.212	0.263
8/16/1996	29.7	6.5	0.20	50.00	28,436	200	0.318	0.309	0.010
8/22/1996	27.4	17.0	0.20	50.00	28,436	8	0.418	0.102	0.316
8/29/1996	30.0	12.5	0.20	50.00	28,436	7	0.500	0.147	0.353
9/5/1996	29.3	13.5	0.20	50.00	28,436	7	0.476	0.135	0.341

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TABLE 2.

continued

Date	T	S	WI	PI	Ck	t _{Crit}	rd	rm	r
9/12/1996	29.7	15.5	1.33	100.00	151,501	1	0.490	0.104	0.386
9/19/1996	28.4	14.5	1.33	100.00	151,501	1	0.447	0.127	0.321
9/26/1996	27.0	17.5	1.33	100.00	151,501	1	0.406	0.099	0.307
10/3/1996	25.1	17.0	1.33	100.00	151,501	2	0.356	0.119	0.237
10/10/1996	22.8	19.5	1.33	100.00	151,501	2	0.304	0.111	0.193
10/17/1996	26.4	20.5	2.30	90.00	636,924	0	0.265	0.051	0.214
10/24/1996	18.4	20.0	2.30	90.00	636,924	0	0.152	0.090	0.062
10/31/1996	26.8	21.5	2.30	90.00	636,924	0	0.272	0.044	0.228
11/7/1996	23.3	24.5	2.30	90.00	636,924	0	0.214	0.045	0.169
11/14/1996	18.8	15.0	2.66	90.00	1,085,308	0	0.070	0.061	0.009
11/21/1996	24.0	16.5	2.66	90.00	1,085,308	0	0.101	0.041	0.060
11/29/1996	14.0	16.5	2.66	90.00	1,085,308	0	0.050	0.054	-0.004
12/5/1996	15.1	13.5	2.66	90.00	1,085,308	0	0.054	0.069	-0.015
12/12/1996	20.1	15.0	2.00	100.00	408,507	0	0.252	0.198	0.053
12/19/1996	6.5	2.5	2.00	100.00	408,507	0	0.025	0.553	-0.528
12/26/1996	11.8	11.0	2.00	100.00	408,507	0	0.142	0.277	-0.135
1/4/1997	20.6	12.0	2.00	100.00	408,507	0	0.261	0.247	0.013
1/9/1997	11.2	6.5	2.00	100.00	408,507	0	0.088	0.399	-0.311
1/16/1997	9.4	6.0	1.60	100.00	225,952	0	0.072	0.416	-0.344
1/23/1997	18.0	14.0	1.60	100.00	225,952	0	0.218	0.217	0.001
1/30/1997	12.3	6.0	1.60	100.00	225,952	0	0.088	0.416	-0.328
2/6/1997	14.9	7.5	1.60	100.00	225,952	0	0.132	0.368	-0.236
2/13/1997	13.0	16.5	0.77	90.00	66,124	1000	0.154	0.177	-0.023
2/20/1997	18.0	13.5	0.77	90.00	66,124	1000	0.218	0.226	-0.008
2/27/1997	18.9	18.5	0.77	90.00	66,124	14	0.232	0.150	0.082
3/6/1997	18.0	6.0	0.77	90.00	66,124	1000	0.131	0.416	-0.285
3/13/1997	21.5	18.5	0.57	90.00	49,178	11	0.277	0.134	0.143
3/20/1997	18.2	4.0	0.57	90.00	49,178	1000	0.088	0.489	-0.401
3/27/1997	23.0	10.5	0.57	90.00	49,178	26	0.308	0.253	0.054
4/3/1997	19.0	11.5	0.57	90.00	49,178	1000	0.233	0.265	-0.032
4/10/1997	19.5	15.0	0.57	90.00	49,178	34	0.241	0.200	0.042
4/17/1997	19.5	5.5	0.80	100.00	69,127	1000	0.133	0.433	-0.300
4/25/1997	19.6	12.0	0.80	100.00	69,127	1000	0.243	0.255	-0.012
5/1/1997	23.4	9.5	0.80	100.00	69,127	40	0.301	0.274	0.027
5/8/1997	24.5	14.5	0.80	100.00	69,127	7	0.342	0.159	0.182
5/16/1997	25.8	6.0	0.53	80.00	46,350	1000	0.224	0.361	-0.136
5/22/1997	27.9	12.0	0.53	80.00	46,350	7	0.432	0.173	0.259
5/29/1997	26.9	6.0	0.53	80.00	46,350	1000	0.242	0.351	-0.109
6/5/1997	27.0	11.5	0.53	80.00	46,350	8	0.406	0.191	0.215
6/12/1997	28.6	13.5	0.80	100.00	69,127	4	0.454	0.140	0.313
6/19/1997	26.7	10.0	0.80	100.00	69,127	7	0.398	0.228	0.169
6/26/1997	28.0	9.5	0.80	100.00	69,127	7	0.413	0.229	0.184
7/3/1997	31.0	9.5	0.80	190.00	69,127	4	0.509	0.204	0.305
7/10/1997	29.7	9.5	0.80	100.00	69,127	5	0.465	0.215	0.251
7/24/1997	29.0	7.5	0.80	100.00	69,127	16	0.350	0.279	0.071
7/31/1997	29.7	17.0	0.80	100.00	69,127	4	0.490	0.087	0.403
8/7/1997	29.4	12.5	0.80	100.00	69,127	4	0.480	0.152	0.328
8/14/1997	30.8	16.0	0.63	70.00	53,746	4	0.528	0.091	0.438
8/21/1997	30.4	14.5	0.63	70.00	53,745	4	0.514	0.112	0.402
8/28/1997	29.0	19.5	0.63	70.00	53,746	4	0.466	0.068	0.399
9/4/1997	29.7	14.5	0.63	70.00	53,746	5	0.490	0.117	0.373
9/11/1997	29.0	19.5	0.63	70.00	53,746	4	0.466	0.068	0.399
9/18/1997	29.9	16.0	1.73	100.00	273,905	0	0.497	0.096	0.400
9/25/1997	26.6	19.0	1.73	100.00	273,905	0	0.395	0.086	0.309
10/2/1997	26.3	13.5	1.73	100.00	273,905	0	0.387	0.159	0.227
10/8/1997	27.2	22.5	1.73	100.00	273,905	0	0.412	0.056	0.356
10/16/1997	17.9	19.0	1.50	100.00	194,859	0	0.216	0.144	0.072
10/23/1997	21.2	21.5	1.50	100.00	194,859	0	0.272	0.106	0.166
10/30/1997	16.3	19.5	1.50	100.00	194,859	0	0.193	0.138	0.055
11/6/1997	19.0	19.0	1.50	100.00	194,859	0	0.233	0.144	0.089
11/14/1997	16.3	14.5	1.84	100.00	322,349	0	0.193	0.208	-0.014

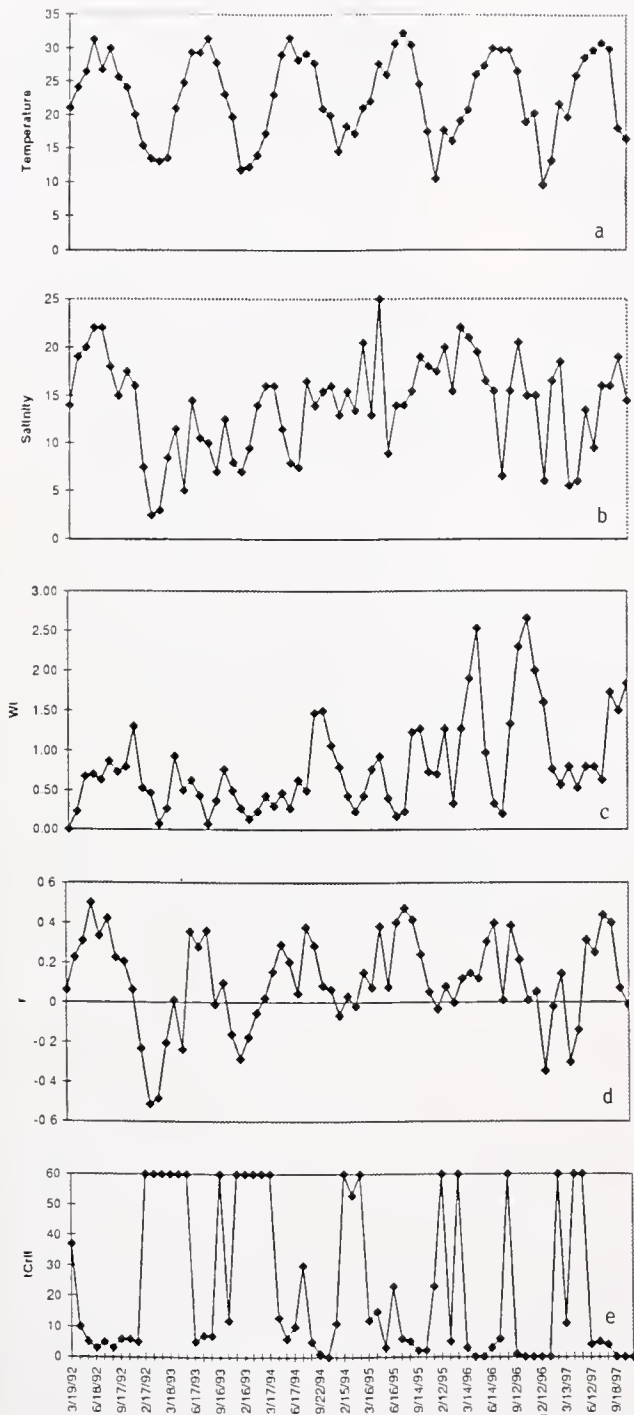


Figure 1. Changes in water temperature (a), salinity (b), WI (weighted incidence of *P. marinus*, c), r (specific rate of change of *P. marinus*, d), and t_{crit} (time to critical level of *P. marinus*, e). Temperature ($^{\circ}\text{C}$), and salinity (ppt) are mid-month values taken when WI was determined, whereas r (day^{-1}) and t_{crit} (days) are calculations for each month generated from the model. Monthly values are plotted for all parameters from March 1992 to November 1997. Complete sample dates and data are given in Table 2.

al. 1995, Powell et al. 1996 does.) Thus, a self-sustaining population of oysters should be less susceptible to catastrophic decline than one that is not self-sustaining (e.g., bedded oysters). Consideration must also be given not only to the t_{crit} value, but also to the

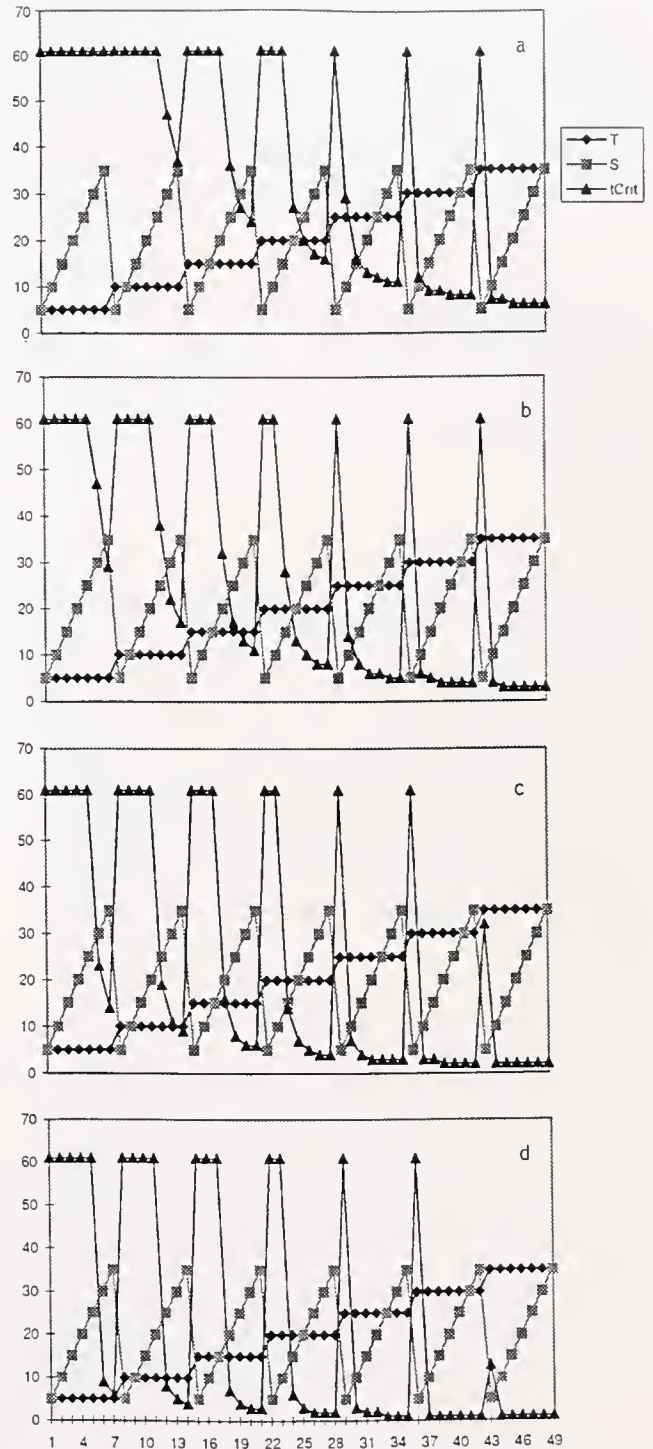


Figure 2. Time to critical level of *P. marinus* (t_{crit}) in days calculated for various combinations of T (temperature in $^{\circ}\text{C}$), S (salinity in ppt) for an initial parasite number C_k . The scale on the y-axis is used for T , S , and t_{crit} . The x-axis is simply an iteration number for a T and S combination input into the model which generates a t_{crit} . Values for initial C_k are 10,000 a), 50,000 b), 100,000 c), and 150,000 d) hyphospores. Calculations are based on an oyster of 3.0 g ash-free dry weight.

time of year from which it was determined. For example, a t_{crit} of a few days in early spring is of much greater concern than the same value in the late fall when water temperatures are rapidly declining.

The model is used to present as a hypothesis which relates temperature, salinity, and initial disease level to time to a critical level of infection. With more frequent measurements of T and S, more frequent estimates of $t_{C_{crit}}$ could be made, improving its reliability. It is our hope that the simple format in which the model is presented, and its modest data requirement, will encourage its testing and application in numerous estuaries.

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RESPONSE OF SETTLING OYSTER LARVAE, *CRASSOSTREA VIRGINICA*, TO SPECIFIC PORTIONS OF THE VISIBLE LIGHT SPECTRUM¹

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ABSTRACT Settlement site choice was used to test the ability of competent-to-settle oyster (*Crassostrea virginica*) larvae to detect specific portions of the visible light spectrum. Larvae were permitted to settle on illuminated or shaded sides of vertically oriented settlement substrates. Five light treatments were used, including white light (400–700 nm), three fractions of white light: red light (600–700 nm), green light (450–575 nm, peak at 525 nm), blue light (400–500 nm, peak at 425 nm); and total darkness. In total darkness, no settlement preference for either side of the substrates was detected. In all light treatments, larvae settled in significantly higher numbers onto shaded surfaces than illuminated surfaces. *Crassostrea virginica* larvae respond to most portions of the visible light spectrum, unlike many previously studied marine invertebrate larvae. This ability may reflect the diverse light conditions in the largely estuarine habitat of this species.

KEY WORDS: *Crassostrea virginica*, larvae, settlement, light

INTRODUCTION

Phototactic behavior in marine larvae can contribute to site selection during the settlement process even though the presence or absence of light is not required for settlement (Baker 1997). Pediveliger (competent-to-settle) larvae of the American oyster, *Crassostrea virginica* (Gmelin), settled primarily on shaded surfaces of substrates in laboratory trials (Ritchie and Menzel 1969). The pediveliger larva of some bivalve mollusks, including oysters (Ostreidae), has a distinct pigmented region termed the *eyespot*, and believed to be photosensitive. Cole (1938) initially described the eyespot and its presumed function for the larvae of the oyster *Ostrea edulis*, although Thompson et al. (1996) point out that research clarifying "eyespot" function is still needed.

Coastal planktonic invertebrates are usually unresponsive, or only weakly responsive, to long visible light wavelengths (orange and red). Serpulid polychaete larvae, which are negatively phototactic when swimming, are responsive to blue and green light (400–550 nm), but are indifferent or respond weakly to orange and red light (>600 nm) (Young and Chia 1982, Marsden 1986, 1988, 1990). Barnacle (*Balanus improvisus* Darwin) nauplii, which are positively phototactic, respond strongly to blue and green light, and also to long-wave ultraviolet (350 nm), but show a marked decrease in response to wavelengths above 600 nm (Lang et al. 1979). The larvae of some estuarine brachyuran crabs, including *Sesarma reticulatum* and *Uca minax*, appear to be most sensitive to light wavelengths of 500–600 nm (green to orange), but sensitivity of most species declines sharply above 600 nm (Forward and Cronin 1979). This phenomenon is not limited to larvae; coastal planktonic adult arrow worms, *Sagitta hispida* Conant, are most sensitive to blue and green light (400–540 nm) but much less sensitive to wavelengths above 520 nm (Sweatt and Forward 1985). The above phototactic responses correlate with spectral attenuation pattern in coastal oceanic waters in which red light (>600 nm) is strongly attenuated, whereas violet to yellow light (400–600 nm) is attenuated the least (Austin and Petzold 1984).

Crassostrea virginica is not primarily an oceanic species, like

most of the above examples, but occurs in greatest abundance in estuaries. The adult and larval life history of *C. virginica* has been reviewed by Stanley and Sellers (1986). In the estuarine environment of the Chesapeake Bay, Virginia, the attenuation of visible light is greatest for short wavelengths (<500 nm), and greatest during the summer when *C. virginica* larvae are most abundant. Yellow and orange light (550–650 nm) generally have the greatest irradiance (transmission) through the water column (Champ et al. 1980, van Tine 1987). The attenuation coefficient (a natural log scale of light reduction with depth) in the Chesapeake Bay during summer is about 3.1 at 400 nm, 1.6 at 500 nm, 1.1 at 600 nm, and 1.4 at 700 nm (van Tine 1987). At a depth of 1 meter, less than 5% of the surface violet light (400 nm) penetrates, but about 30% of the yellow and orange light (550–650 nm) is still present. Water quality, including inorganic and organic particulate matter, strongly affects light quality in estuarine waters (Pierce et al. 1986). It is probable, therefore, that *C. virginica* larvae experience a wide range of light quality within a single estuary.

If oysters are unresponsive to certain portions of the visible light spectrum, as are the larval species discussed above, then water quality parameters that affect light quality (Pierce et al. 1986) could affect the ability of oyster larvae to use light as a settlement cue. If oyster larvae do not respond to red and orange light wavelengths, then much of the Chesapeake Bay would be a light-poor environment to larval oysters (van Tine 1987). This study addresses the question: Do larvae of oysters (*C. virginica*) respond to different portions of the visible light spectrum, as observed by settlement site choice?

MATERIALS AND METHODS

Oyster larval settlement chambers were constructed from 1/8 inch (3 mm) thick black acrylic Plexiglas. One side was made of clear acrylic to permit light entry. Each chamber had internal dimensions of 7.5 cm in height, 7.5 cm in width, and 2.5 cm in depth, and a volume of about 60 ml. A clear acrylic bracket was placed in the bottom of each chamber, at least 2 cm from the sides of the chamber (Fig. 1), to hold a settlement substrate plate in a vertical position. The vertical orientation of the settlement plate was used to eliminate geotaxis (Pires and Woollacott 1983, Baker 1997) and barokinesis (Crisp and Ghobashy 1971, Mann and Wolf 1983) as confounding settlement cues.

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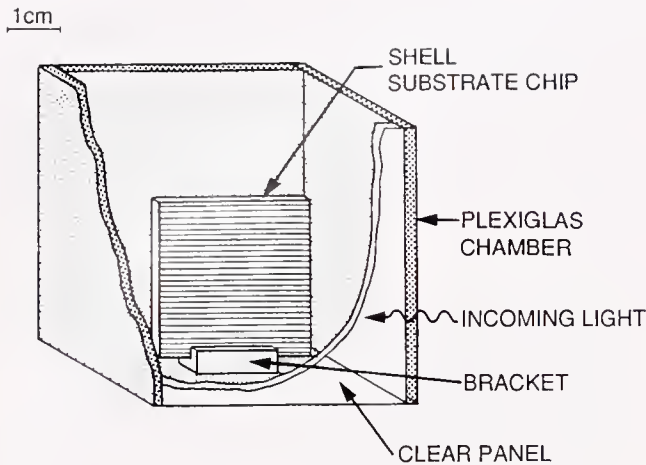


Figure 1. Diagram of a settlement chamber.

Settlement substrate plates were cut from adult oyster (*Crassostrea virginica*) shells, 3 × 3 cm square by 2 mm thick, and ground to equal roughness on both sides. Settlement plates were placed in seawater two days prior to use, to allow bacterial growth on the shell surfaces; water-soluble compounds from oyster shell and some levels of bacterial colonization induce settlement in *Crassostrea* (Hidu et al. 1978, Fitt et al. 1990). The tops of each chamber were covered with a black acrylic plate to reduce evaporation, but were not airtight.

The light source used for this study was a pair of new 48-inch Philips cool white fluorescent tubes. About 95% of the light output of the fluorescent lamp was between 400 and 700 nm, based on specifications provided by the manufacturer. The light source was fixed at 25 cm from the settlement chambers (chambers were arranged in rows facing the fluorescent tubes). Total visible light flux from the fluorescent lamp was measured with a Li-Cor radiometer-photometer, Model LI-185A, using an on-deck light sensor. Neutral density black scrim was used to regulate light intensity. Light entering the settlement chambers was 2.4–2.6 microEinsteins ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), after all filters were in place. This range is equivalent to midsummer light intensity at about a 5 m depth in the lower Chesapeake Bay, using a light attenuation coefficient (k) of 1.1, and assuming surface insolation of 500 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Wetzel and Neckles 1986). Light levels used in similar trials by Ritchie and Menzel (1969) were 25–50 foot candles (≈ 5.2 – $10.4 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

Five light treatments were used: white, red, green, blue, and total darkness. White light was unfiltered from the fluorescent lamp. The total darkness treatment consisted of settlement chambers made entirely of black acrylic, which blocked all light, with no clear acrylic panel. For red, green, and blue light, theatrical light filters (Rosco and Lee brands) were used. Transmittance spectra of filters were measured with a Shimadzu spectrophotometer, Model UV 160. The red filter was Roscolux Medium Red overlaid with Lee Orange; this combination transmitted wavelengths only above 600 nm. The green filter was Roscolux Dark Yellow Green (470–600 nm, peak at 525 nm), and the blue filter was Lee Deep Blue (380–525 nm, peak at 425 nm). Both green and blue filters transmitted some far red (above 740 nm) light, but these wavelengths were mostly above the fluorescent lamp output. Light filters and black scrim were attached directly to the outside of the settlement chambers.

Competent-to-settle pediveliger larvae of *Crassostrea virginica* were reared at 20 ppt salinity in the Virginia Institute of Marine Science oyster hatchery. Approximately 500 larvae, in 55 ml of 20 micron-filtered seawater (20 ppt), were pipetted into each settlement chamber. A fan was used to circulate air past the settlement chambers, and temperature within the chambers remained at 25–28°C throughout the trial.

All settlement trials ran simultaneously for 24 h at constant light conditions. There were six settlement chambers for each color treatment, and each series of trials (five treatments) was run twice, for a total of 12 replicates per treatment. At the end of each trial, settled juveniles (spat) on the illuminated ("front") and shaded ("back") surfaces of each substrate plate were recorded. Settlement on each side (illuminated and shaded, or front and back in the case of the no-light treatment), was expressed as a proportion of total settlement for each substrate plate. The difference between proportional settlement onto shaded and illuminated surfaces was calculated for each replicate substrate.

Paired-sample *t*-tests were used to test the null hypothesis that the mean proportional difference between illuminated and shaded (front and back) for each light treatment was equal to zero (Zar 1996). Prior to analysis, the absolute of each difference was transformed using the arcsine-square root transformation (Zar 1996), and then converted back to its original sign.

RESULTS

In total darkness, no significant settlement difference between front and back surfaces of the settlement substrate plates was detected. In all other treatments, proportional settlement of *Crassostrea virginica* was significantly higher onto shaded sides of settlement plates. Results are summarized in Table 1.

Larval mortality was less than 1% in any treatment, and larvae that had not settled were still swimming. About 10–12% of the larvae in each trial settled, which was typical for hatchery-reared *C. virginica* larvae at that time (Baker 1994).

DISCUSSION

Competent-to-settle larvae of *Crassostrea virginica* respond to red, green, and blue portions of the visible light spectrum in the same manner as they do to white light. In this regard, they are unlike many previously studied coastal larvae, which appear to be unable to use orange or red light (Lang et al. 1979, Young and

TABLE 1.

Summary of differences of settled *Crassostrea virginica* between back (shaded) and front (illuminated) surfaces. Values are given for mean proportion of larvae settled on shaded (Back) surfaces; the means (Δ Mean) and standard deviations (Δ STDS) of the differences between proportions on shaded and illuminated surfaces, and type I error probabilities (*p*) from one-sample *t*-tests (based on arcsine-square root data transformations).

Treatment	Total Darkness	Blue Light	Green Light	Red Light	White Light
Back	0.483	0.783	0.792	0.711	0.743
Δ Mean	–0.035	0.565	0.583	0.422	0.485
Δ STDS	0.513	0.265	0.346	0.267	0.309
<i>p</i>	0.9467	<0.0001	0.0001	<0.0001	0.0001

Chia 1982, Marsden 1986, 1988, 1990). Coastal waters that transmit little usable light to other species still transmit sufficient light in the longer wavelengths to be a settlement cue for *C. virginica* larvae.

The breadth of the spectral responsivity of *C. virginica* larvae reflects the range of water column conditions this species encounters. The spectral transmittance of sea water is strongly modified by dissolved and particulate terrigenous matter, and thus varies between and within estuaries (Pierce et al. 1986). The adult habitat of *C. virginica*, and thus the habitat of competent-to-settle larvae, ranges from oceanic to seasonal salinities as low as 5‰ (Wells and Gray 1960, Stanley and Sellers 1986). A broad spectral responsivity would be a beneficial adaptation for a species occupying this range of habitats. The brine shrimp, *Artemia salina*, also has a broad spectral sensitivity, and also occupies highly variable water column conditions (temporary ponds), although peak sensitivity appears to be below 600 nm (Aiken and Hailman 1978).

On the other hand, light is not a required settlement cue for *C. virginica*. In both this study and others (Richie and Menzel 1969, Baker 1997), *C. virginica* larvae settled in the total absence of light. Gravity appears to be a strong settlement cue by itself. Either light avoidance or geotaxis could permit larvae to settle on lower

surfaces of adult oyster shells in the field, but the settlement patterns that result are equally marked in darkness (Baker 1997), indicating gravity as a sufficient cue. Gravity is a constant, light is not. Furthermore, other cues are also available: *C. virginica* has been shown to settle in response to water-borne chemicals from conspecifics (Hidu et al. 1978), and *C. gigas*, a similar species, settles in response to chemicals produced by certain bacterial conditions on the substrate (Fitt et al. 1990).

The question then arises: Why has *C. virginica* evolved a phototactic response during settlement? Assuming that the eyespot is, in fact, a photosensory organ (Thompson et al. 1996), why does it develop only in the competent-to-settle larvae?

One possibility is that phototaxis permits a "fine-tuning" of the settlement response. Oysters, unlike most bivalve mollusks, cement permanently to the substrate immediately upon settlement, and cannot subsequently adjust their habitat choice (Kennedy 1996). Laboratory studies deliberately reduce variables and provide larvae with clear choices (up-down, light-dark), but natural ecosystems are likely to be more complex. It is, therefore, probably of selective advantage to invest in additional sensory systems to gain as much information as possible about a potential permanent home.

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RECRUITMENT PATTERNS OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, ALONG A CREEK GRADIENT IN HOUSE CREEK, LITTLE TYBEE ISLAND, GEORGIA

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ABSTRACT Our goal is to produce large, deep-cupped, single oysters, *Crassostrea virginica*, for the half-shell and steamer market. However, oysters in Georgia are small and grow in clusters because of high recruitment rates and lack of appropriate substrate upon which oyster spat can settle. It was hypothesized that oyster recruitment would be lower at sites farther back into a tidal creek presumably because of high tidal flushing rates (40% exchange per tide). To test this hypothesis, spat collectors were deployed monthly at 10 sites along two branches of House Creek, Little Tybee Island, Georgia from April to November, 1996. The total number of oysters on each collector was enumerated and 30 oysters per site were measured for shell length. Significant differences in recruitment rates were observed between sites ($p < .001$). Generally, oyster spat recruitment was lowest at the most remote sites in a tidal creek and increased seaward. The recruitment rate at one site was higher than expected based on our hypothesis. However, the percent cover by adult oyster reefs at this site was also higher and may explain the higher recruitment rate. Therefore, our data supports the hypothesis. Based upon the results of this study, the most remote sites in a tidal creek with relatively low percent cover by adult oyster reefs should exhibit low recruitment rates. It may therefore be possible to reduce oyster clustering by culturing oysters in these areas for grow-out to market size.

KEY WORDS: Oysters, aquaculture, fouling, recruitment, *Crassostrea virginica*, spatfall

INTRODUCTION

Georgia has 450,000 acres of salt marsh, most of which is undeveloped and relatively unpolluted. Given this, and the ideal environmental conditions of salinity (10 ppt–30 ppt) and water temperature (8–30°C), the Georgia coast has the potential to be a prime location for Eastern oyster, *Crassostrea virginica* (Gmelin), aquaculture (Helferman and Walker 1988). Due in part to a large tidal amplitude (2.4–3 m), oyster population dynamics are unique in that oysters in Georgia occur mostly in the intertidal zone (Harris 1980). Therefore, oyster culturing techniques used successfully in areas where oysters occur subtidally might not be as effective in Georgia. Specific culturing techniques need to be developed for Georgia that account for the unique environmental characteristics.

Dame et al. (1984) noted that oyster reefs reach their greatest biomass and density in the southeastern United States, in particular South Carolina and Georgia. However, the oysters are relatively small and tend to grow in clusters, making harvesting and subsequent handling difficult. Also, high recruitment rates and the lack of appropriate substrate for setting cause oysters to settle upon and compete with each other. This results in overcrowding such that oysters only have room for lateral growth. Oysters grown in such conditions are long, narrow, and shallow, and the meat is of little commercial value (Adams et al. 1991).

Eighteen months of growth are required for an oyster to attain legal market size in Georgia (Helferman and Walker 1988). Thus, oysters must grow through at least one spawning cycle. May through October (Helferman et al. 1989), where oyster spat (juvenile oysters that have successfully recruited) may settle upon cultured oysters. O'Beirn et al. (1995) determined that oyster recruitment in Georgia can be higher than recruitment rates observed in other parts of the southeastern United States. Mean monthly recruitment rates as high as 35,000 spat/m² have been documented (O'Beirn et al. 1994). The degree of fouling by oyster spat presents a serious problem for oyster aquaculture in this region.

It may be possible to control the degree of fouling by growing

oysters to market size in areas of low oyster recruitment. Therefore, identifying areas of low recruitment would be useful information in developing aquacultural techniques. We hypothesized that oyster recruitment would be lower at sites farther back into a tidal creek presumably because of high tidal flushing rates. The hypothesis of this experiment was based upon the results of a two-yr study of oyster recruitment conducted by O'Beirn et al. (1997) in the Duplin River, Sapelo Island, Georgia. They found that areas farther back in this tidal river had lower recruitment rates which may be attributed to the larvae being flushed out of these areas during tidal changes. This is a possibility because Ragotzkie and Bryson (1955) showed that a 40% water exchange occurred with each tidal cycle for the Duplin River. Therefore, the most remote locations in tidal creeks might be the best for oyster grow-out because oyster spat fouling might be reduced by larvae being washed out with the tide.

This study monitored the temporal and spatial patterns of recruitment, spat size, and water temperature at 10 sites in House Creek. Identifying spatial recruitment patterns will enable us to test the hypothesis. Studying temporal and spatial patterns of recruitment, spat size, and water temperature will provide important information in developing oyster aquacultural techniques.

Site Description

This study was carried out in House Creek which is part of the Little Tybee Island complex located just outside Savannah, Georgia (Fig. 1). The area is predominated by salt marsh and small tidal creeks. Wassaw Sound is the primary source of water for both branches of House Creek utilized in this experiment. One branch of the creek is connected to another water source, the Bull River, at high tide and the other branch is a dead end. Under our hypothesis, sites closest to Wassaw Sound and the Bull River should have higher recruitment rates than the sites farthest back into the creek. In addition, we expected to observe a gradient of recruitment rates from sites farthest back in the creeks seaward. In this case, cul-

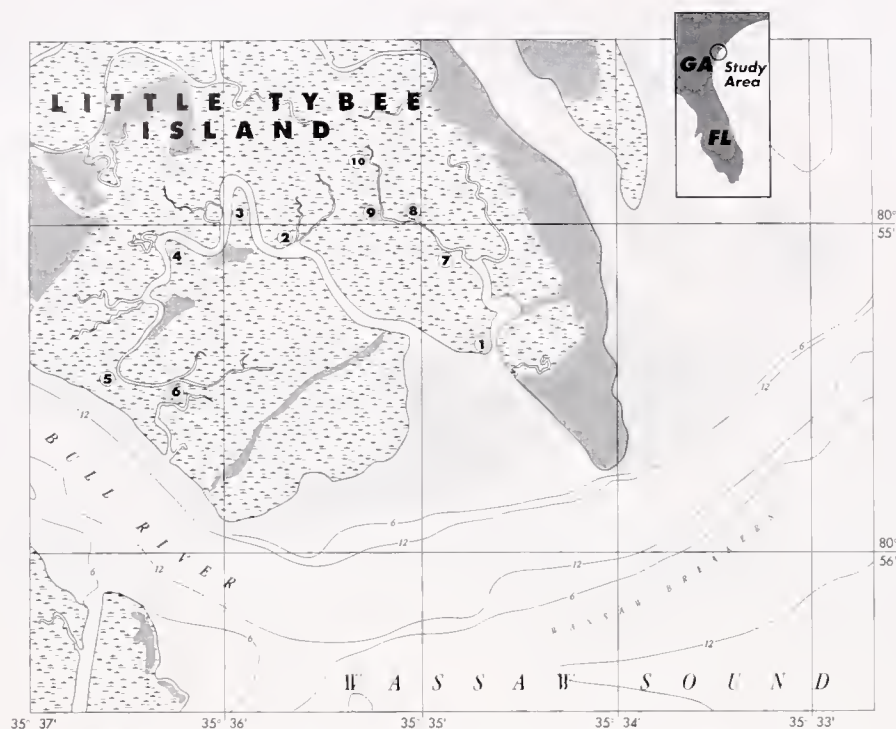


Figure 1. Oyster spat collection sites in House Creek, Little Tybee Island, Wassaw Sound, Georgia.

turing methods could be employed so that spat could be collected from areas of highest recruitment and transplanted to areas of low recruitment for growout to market size.

METHODS

To test our hypothesis, 10 sites were chosen along both branches of House Creek: one where the creek branches, five along the larger branch that is influenced by the Bull River, and four along the second branch (Fig. 1). Rebar frames were permanently installed at each site to ensure that the collectors were positioned at mean low water, near a living oyster reef, and approximately 15 cm off the shell bottom. The collectors were gray PVC pipes (2 cm in diameter, cut in 15-cm segments, of which 12.5 cm was used in the analysis, which resulted in a total surface area of 0.01 m²) with longitudinal grooves and embedded calcium chips. The four collectors were placed on PVC frames designed to fit on the rebar frames (Fig. 2). Prior to deployment, the collectors were conditioned in a raceway with running sea water for several days.

The study was initiated on 27 March 1996 at which time the first set of collectors was deployed at each site. The collectors were replaced with a new set on a monthly basis and the study was terminated on 24 November 1996. After the deployment period, the collectors were lightly rinsed with fresh water to remove any loose debris and oyster larvae that were not fully cemented to the substrate. For the purpose of this study, we defined oyster spat as oysters that had metamorphosed, cemented, and exhibited a fan of shell growth. Therefore, larvae that were washed away because they had not fully cemented were not considered spat.

On a monthly basis, all the oysters on each collector were counted using a dissecting microscope (10 \times). In addition, 30 oysters were measured for shell length (umbo to lip) per site. When less than 30 spat settled per site, all oyster spat were measured. Oysters smaller than 10 mm were measured with an ocular mi-

crometer in the microscope. Oysters larger than 10 mm were measured using Vernier calipers.

Optic StowAway Temperature Loggers from Onset Computer Corporation were deployed at each site. The probes were placed in plastic bags with holes and then placed inside PVC pipes with holes to inhibit biofouling. These units were weighted down with a brick and attached to the rebar frame using heavy fishing line. The water temperature loggers were programmed to take measurements every six min. The data were downloaded to the Optic Shuttle and then directly into the computer via the Optic Base Station on a monthly basis.

The areas occupied by the adult oyster reefs were estimated by multiplying the longest length by the longest perpendicular width of each reef within a 30.5 m radius of the collectors. The resulting number was divided by the area of a circle with a 30.5 m radius and multiplied by 100% to get the percent cover by the adult oyster reefs at each site. The oyster reefs included shell as well as living oysters. These data were collected in March 1998. It is possible that the areas occupied by the oyster reefs changed during the interval between the end of the recruitment study and the collection of this data. However, this was intended to provide an estimate only, and based upon personal observation it is unlikely that the areas changed enough to substantially effect the estimate.

The raw monthly recruitment data were converted to the average number of spat per collector ($n = 4$) at each site and then analyzed using Friedman's Method for Randomized Blocks. First, months were used as blocks ($b = 6$) and then sites were used as blocks ($b = 10$) to determine if there were differences in recruitment between sites and months, respectively. The raw monthly size data were converted to the average spat size ($n = 30$, when available) at each site and then analyzed using Friedman's Test, again blocking months ($b = 6$) and sites ($b = 10$), independently (Conover 1980). The purpose for deploying collectors during the

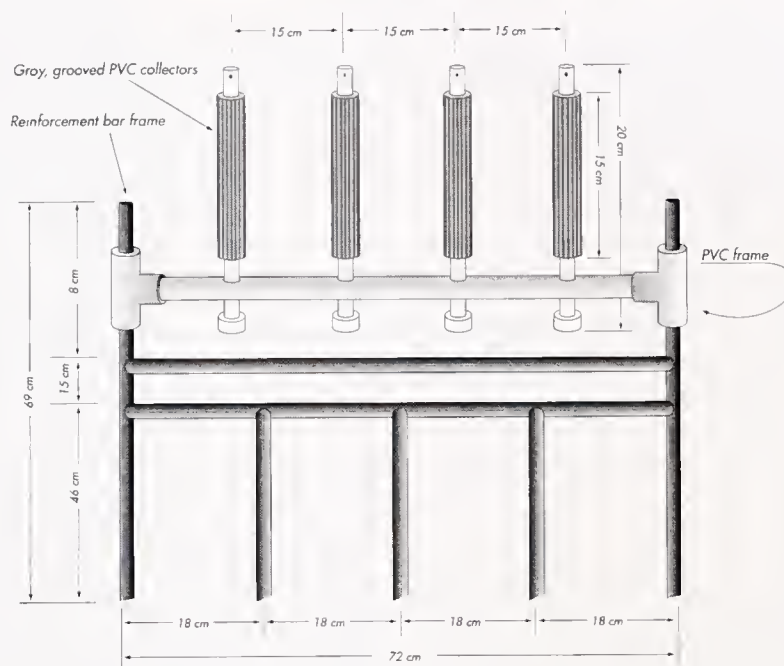


Figure 2. Spat collectors attached to an exchangeable PVC frame mounted on a permanent frame on site made of rebar.

months of April and November was to ensure that we did not miss the beginning and end of the recruitment period. We did not expect to observe recruitment during those months, although it is possible. Therefore, only those months in which recruitment was actually observed were used in the analysis of the recruitment and size data. In October, although no recruitment was observed at sites 2, 4, and 8, they were still used in the analysis and assigned the lowest rank in their blocks. If significant differences were detected, the multiple comparisons test suggested by Conover (1980) was used to determine which sites or months were significantly different from each other.

Daily ($n \approx 225$) and biweekly ($n \approx 3100$) water temperature averages were obtained from the raw data at each site. Monthly water temperature averages were calculated as the mean of the daily water temperature averages ($n \approx 30$) at each site. The daily water temperature averages ($n \approx 30$) were analyzed using one-way analysis of variance (ANOVA) to determine if there were significant differences in water temperature between sites for each month. The test was conducted with equal sample sizes for April, July, and August, and unequal sample sizes for the remaining months because the temperature probes failed at several sites. If differences were observed, a Tukey-Kramer test was used to determine which sites were significantly different from each other (Sokal and Rohlf 1995). The statistical package used for the ANOVA and Tukey-Kramer tests was JMP (SAS Institute 1989).

RESULTS

The average number of spat per collector ($n = 4$) at each site is plotted over time in Figure 3. Recruitment was first observed in May at all 10 sites. All sites demonstrated peak recruitment rates in June and another small peak was observed in September at most sites. Recruitment ended at sites 2, 4, and 8 in September, whereas the other seven sites last exhibited recruitment in October. Sites 1 and 2 demonstrated the largest peaks, with means of 457 and 650 spat/0.01 m², respectively, and had a total of more than 2,000 spat

settle during the season. The lowest recruitment rates were observed at sites 4 and 10, neither of which exhibited a mean of more than 10 spat/0.01 m² during any time period or a total of more than 100 spat settling during the entire season.

There were significant differences in the average number of spat per collector ($n = 4$) between sites when months were blocked (Friedman, $p < 0.001$). Table 1 shows the results of the multiple comparisons test to determine which sites were significantly different. Sites 1, 7, and 9 were ranked the highest in re-

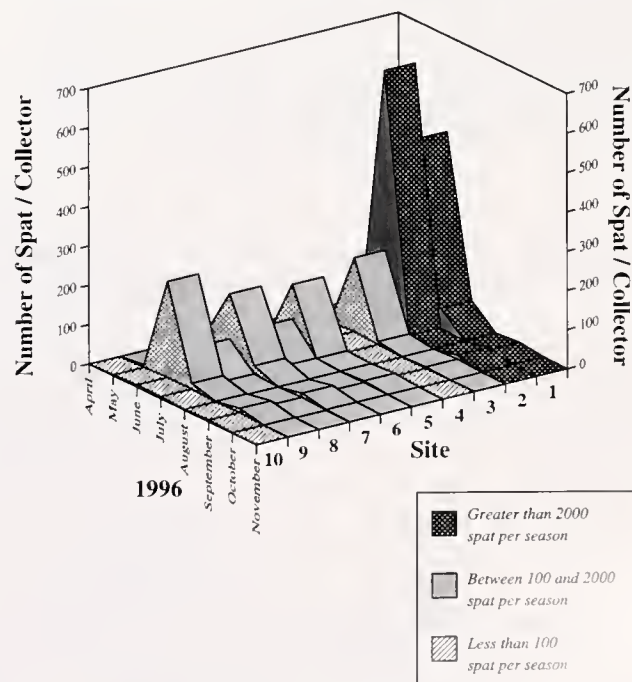


Figure 3. Mean number of spat collected per month per site for the ten sites in House Creek, Little Tybee Island, Georgia.

TABLE 1.

The results of the multiple comparisons test on the average recruitment data to determine which sites were significantly different.

Ranking of Recruitment by Site Multiple Comparisons $p = 0.05$										
	High → Low									
Site:	1	7	9	2	5	6	3	10	8	4

This test was warranted because the Friedman test on the average number of spat per collector ($n = 4$), using months as blocks, resulted in significant differences ($p < 0.001$) between sites. Only months in which recruitment was observed were used in the Friedman test. Sites underlined by the same line are statistically the same.

cruitment and were significantly higher than sites 8 and 4, which were ranked the lowest. However, there is a high degree of overlap of significance based on this test.

A summary of the rankings of the total number of spat settling during the season, the results of the multiple comparisons test on the average recruitment data, and the percent cover by adult oyster reefs at each site is presented in Table 2. The ranking of the total number of spat settling during the season is based upon Figure 3. Table 1 is used to rank the sites according to the results of the multiple comparisons test on the average recruitment data. Sites where the percent cover by adult oyster reefs was greater than 10% were ranked high, between 5 and 10% were ranked medium, and less than 5% were ranked low. The location of each site along both branches is also described. Site 1 is considered part of both branches because it is located where House Creek bifurcates.

When sites were blocked, there were significant differences in the average number of spat per collector ($n = 4$) between months (Friedman, $p < 0.001$). The results of the multiple comparisons test to determine which months were significantly different are shown in Table 3. Each month was significantly different in recruitment

from the rest, with the exception of May and September which were statistically the same. June was ranked the highest in recruitment and October was ranked the lowest.

The average spat size ($n = 30$ when available) at each site is plotted over time in Figure 4; it ranged from 0.37 mm to 4.92 mm. Spat size was highly variable within sites. However, the data seem to exhibit a general trend: a peak in spat size occurred in June, followed by another peak in August or September.

When sites were blocked, significant differences in the average spat size ($n = 30$ when available) were observed between months (Friedman, $p < 0.001$). The results of the multiple comparisons test to determine which months were significantly different are shown in Table 4. August and September were statistically the same in ranking of oyster size, as well as October and May. All other comparisons were significantly different. June was ranked the highest in spat size and October and May were ranked the lowest. When months were used as blocks on this data, there were no significant differences between sites (Friedman, $p > 0.05$).

The average oyster recruitment versus water temperature is plotted in Figure 5. The average oyster recruitment is the average number of spat per collector ($n = 4$) per month and site. The water temperature is the monthly average at the corresponding site. There were several sites where the temperature probes failed to collect data during the entire month of collector deployment. These sites were not included in this plot. Oysters settled within a water temperature range of 23.5–29.0°C. Maximum recruitment occurred at a water temperature of 27.5°C. Heaviest recruitment occurred in June when water temperatures increased to 27°C, rather than in September when water temperatures decreased to 27°C (Figs. 3 and 6). However, a small peak in oyster recruitment was noted in September.

Figure 6 is a plot of the water temperature over time throughout the recruitment season. The water temperature is the average of the biweekly water temperature averages at all 10 sites. On 16 April, sites 8 and 9 had significantly higher water temperatures than the other eight sites (Table 5); therefore, those two sites were plotted separately on that date. The water temperature rose steadily between 16 April and 26 June 1996. Between 26 June and 20 August, the water temperature leveled off at about 28.5°C. Following 20 August, the water temperature decreased to a low of about 14.5°C in December.

TABLE 2.

A summary of the rankings of the total number of spat settling during the season, the results of the multiple comparisons test on the average recruitment data, and the percent cover by adult oyster reefs at each site along both branches. The location of each site along both branches is described.

Branch	Site	Location Along Branch	Number of Spat	Multiple Comparisons	Percent Cover
Large	1	Most seaward	h	h	h
	2	↓	h	m	m
	3		m	m	l
	4		l	l	l
	5	Most remote	m	m	h
	6	Close to Bull River	m	m	h
Small	1	Most seaward	h	h	h
	7	↓	m	h	h
	8		m	l	l
	9		m	h	h
	10	Most remote	l	l	l

Site 1 is considered part of both branches because it is located where House Creek bifurcates.

Key: (h)high: >2000 spat; Sites 1, 7 & 9; >10%; (m)medium: 100–2000; 2, 5, 6, 3 & 10; 5–10; (l)low: <100; 8 & 4; <5.

TABLE 3.

The results of the multiple comparisons test on the average recruitment data to determine which months were significantly different.

Ranking of Recruitment by Month Multiple Comparisons $p = 0.05$						
High —————→ Low						
Month:	<u>June</u>	<u>July</u>	<u>May</u>	<u>September</u>	<u>August</u>	<u>October</u>

This test was warranted because the Friedman test on the average number of spat per collector ($n = 4$), using sites as blocks, resulted in significant differences ($p < .001$) between months. Only months in which recruitment was observed were used in the Friedman test. Months underlined by the same line are statistically the same.

The results of the ANOVA and Tukey-Kramer tests performed on the daily water temperature averages ($n = 30$) are shown in Table 5. Significant differences in water temperature were observed between sites (ANOVA, $p < 0.05$) during the months of April, August, November, and December. Of those, the only month during which oyster recruitment was observed was August. However, the Tukey-Kramer test ($p = 0.05$) was unable to detect significant differences in water temperature between the sites during August. During April, sites 8 and 9 were significantly higher in water temperature than all the other sites (Tukey-Kramer, $p = 0.05$). In November and December, the Tukey-Kramer test ($p = 0.05$) was able to detect significant differences in water temperature between sites, however, there is a high degree of overlap.

DISCUSSION

In general, oyster recruitment began in May, peaked in June, demonstrated another small peak in September, and terminated in October (Fig. 3). When sites were blocked, there were significant differences in the average number of spat per collector between months (Friedman, $p < 0.001$, Table 3). June was ranked the highest month which corresponds to the peak recruitment period in June. Since another small peak was observed in September, it would be expected that September should be ranked second. However, the recruitment rate in July was higher than September, but July was exhibiting a decreasing trend and was therefore not considered a peak. These general seasonal patterns were similar to those observed in this region by other authors (Kenny et al. 1990, McNulty 1953, O'Beirn et al. 1996).

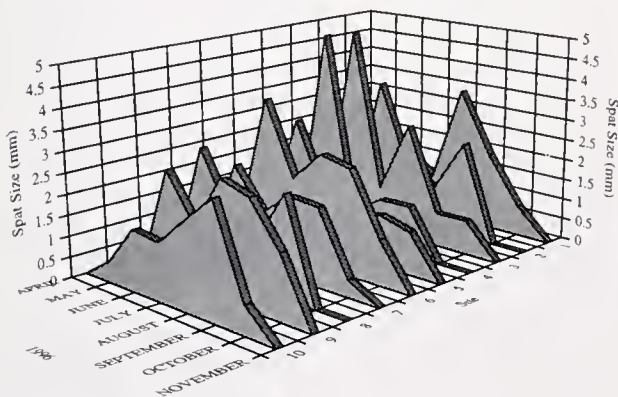


Figure 4. Mean spat shell length in mm of oysters collected per site per month from House Creek, Little Tybee Island, Georgia.

TABLE 4.

The results of the multiple comparisons test on the average spat size to determine which months were significantly different.

Ranking of Spat Size by Month Multiple Comparisons $p = 0.05$						
Large —————→ Small						
Month:	<u>June</u>	<u>August</u>	<u>September</u>	<u>July</u>	<u>October</u>	<u>May</u>

This test was warranted because the Friedman test on the average size of 30 spat (when available), using sites as blocks, resulted in significant differences ($p < 0.001$) between months. Only months in which recruitment was observed were used in the Friedman test. Months underlined by the same line are statistically the same.

More than 8,000 spat settled in June (the sum of all sites and collectors), whereas less than 50 spat settled in October. These results support the findings of Kenny et al. (1990) in that the within year variability in recruitment numbers may be high. In addition, Kenny et al. (1990) noted that these temporal patterns are fairly consistent among years for each geographic location, and that the peak recruitment periods coincide with the spawning patterns of the local adults. Yet, O'Beirn et al. (1997) found that spawning events were not necessarily followed by peaks in recruitment. This emphasizes the point that recruitment is dependent upon many factors, which include rate of gametogenesis and spawning, water temperature, salinity, food availability, tidal flushing, etc. Perhaps, in the year O'Beirn et al. (1997) conducted the study, these other factors had more important roles in determining recruitment rates.

Based upon our hypothesis, we expected to observe the highest recruitment rate at site 1 (the site closest to Wassaw Sound). Along the small branch of House Creek we expected a gradient of recruitment rates, the lowest at site 10 and increasing seaward. The

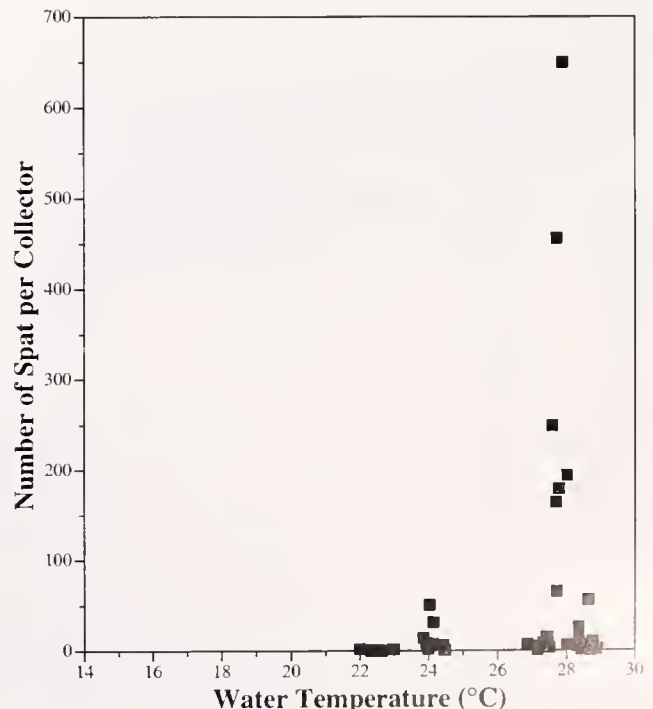


Figure 5. The relationship between water temperature and the mean number of spat collected from House Creek, Little Tybee Island, Georgia.

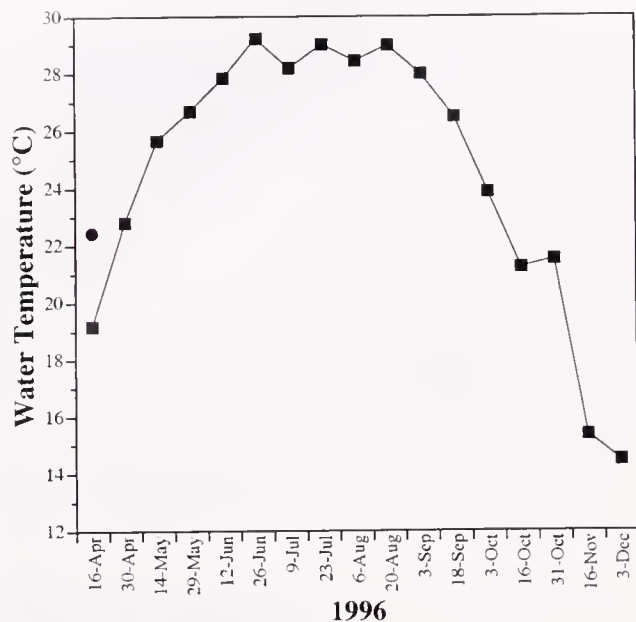


Figure 6. The mean water temperature in House Creek, Little Tyhee Island, Georgia. Significant differences in water temperature was detected only at sites 8 and 9 on April 16, thus the separate data point graphed on that date.

large branch is influenced by the Bull River at site 5 at high tide. Along the large branch we expected site 1 to be the highest, the sites closest to the Bull River (sites 5 and 6) to be the next highest, and a gradient between sites 1 and 5. Consistent with our hypothesis, both the number of spat settling and the multiple comparisons test revealed that recruitment at site 1 was significantly higher. In fact, the number of spat settling is consistent with our hypothesis

TABLE 5.

The results of the analysis of variance on the daily water temperature averages ($n = 30$) to determine if there were significant differences between sites for each month.

Month	ANOVA p-Value	Ranking of Temperature by Site per Month Tukey-Kramer $p = 0.05$									
		High → Low									
April	0.0001*	8	9	6	4	3	10	2	7	5	1
May	0.4926 ^{ns}										
June	0.6700 ^{ns}										
July	0.2423 ^{ns}										
August	0.0394*	No differences									
September	0.0912 ^{ns}										
October	0.2138 ^{ns}										
November	0.0313*	6	8	7	4	1	3	5	10	2	x
December	0.0001*	6	8	7	4	2	3	1	10	5	x

Where significant differences were observed, the Tukey-Kramer test was used to determine which sites were significantly different from each other. Sites connected by the same line are statistically the same.

ns = not significant; * = significant; x = site 9 had no data points and was excluded from the analysis.

at all sites. However, the multiple comparisons test indicates that site 9 is the only site that is inconsistent with our hypothesis (Table 2). This anomaly may be explained by the percent cover by adult oyster reefs. Metamorphosed oysters are believed to emit pheromones which stimulate larvae to set (Hidu and Haskin 1971). The percent cover by adult oyster reefs at site 9 ranked high at 12.7% (Table 2). With greater numbers of adult oysters at site 9, the concentration of pheromones may be high enough to cause a greater number of larvae to set. Therefore, our data support the hypothesis of oyster spat recruitment being the lowest at the most remote sites and increasing seaward.

This experiment identified two areas of low recruitment, sites 4 and 8 (Table 1). It is possible that the factors that cause lower recruitment rates at these sites could also affect the growth and survival of the oysters placed in these areas for growout to market size. However, there were no significant differences between sites for spat size when months were blocked (Friedman, $p > 0.05$). Consequently, factors causing lower recruitment rates do not significantly affect the growth of the spat. In addition, it is likely that the larger spat transplanted to low recruitment areas for growout will be more resistant to factors causing lower recruitment rates than oyster larvae and newly settled spat. Therefore, the spat could be collected from high recruitment areas (sites 1, 7, and 9) and transplanted to low recruitment areas (sites 4 and 8) for growout.

The spat size data, as illustrated in Figure 4, appear to exhibit a general trend: a peak in spat size in June, followed by another peak in August or September. There were significant differences in average spat size between months when sites were blocked (Friedman, $p < 0.001$, Table 4), which substantiates this observation. The largest spat were measured on the June, August, and September collectors in which case oysters from the June sample were significantly larger than those measured in August and September. These peaks may be explained by the time at which the larvae settle. For instance, if the larvae settled when the collectors were first deployed, they would have an entire month to grow. Identifying sites or time periods with the best spat growth would be very useful information from an aquacultural perspective. However, the temperature, salinity, quality of food, and many other factors make growth rate difficult to predict. Further studies would need to be conducted to determine if time of deployment affects the spat size.

Water temperature has been shown to act as a stimulus that induces setting behavior in oysters (Hidu and Haskin 1971). The average oyster recruitment versus water temperature plot (Fig. 5) shows that the majority of setting occurred between 23.5 and 29.0°C with a peak at 27.5°C. The peak biweekly water temperature average for House Creek in 1996 was 29.2°C, consistent with the findings of O'Beirn et al. (1996) in 1991, 1992, and 1994. In 1993, O'Beirn et al. (1996) noted higher than usual water temperatures ranging from 29.2 to 31.5°C which coincided with lower than usual recruitment rates observed on the biweekly and monthly collectors. They suggested that these lower recruitment rates might be explained by the observed temperatures exceeding the upper temperature tolerance for oyster larvae. During the months in which oyster recruitment was observed, there were no significant differences in temperature between sites (Table 5). Therefore, the differences between sites in recruitment rates cannot be attributed to different water temperatures.

An interesting observation about the recruitment (Fig. 3) and temperature (Fig. 6) data was that both peak recruitment periods occurred when the temperature was around 27°C. In June, the temperature was increasing to 27°C and in September it was de-

creasing to 27°C. This might suggest that 27°C is an optimum temperature for oyster recruitment. However, additional studies would need to be conducted to verify this.

Our goal is to produce large, deep-cupped, single oysters for the half-shell and steamer market in Georgia. A quality product may be obtained by reducing the degree of clustering and providing the spat with the potential for fast growth. By deploying spat collectors in July or August, after the peak recruitment period, fouling by additional spat would be considerably reduced because the majority of setting would have already occurred. This, coupled with the larger spat observed in August and September, would provide the newly settled spat with fast growth potential and reduced crowding.

Since 18 months of growth are required for oysters to reach market size in Georgia (Heffernan and Walker 1988), the cultured oysters would then be exposed to additional oyster spat fouling during the second recruitment season. However, culturing oysters in areas of low recruitment would further reduce such fouling

without adversely affecting the growth potential. Based upon the results of this study, the most remote sites in a tidal creek with relatively low percent cover by adult oyster reefs should exhibit low recruitment rates. In addition, biofouling during the recruitment season can further be reduced by culturing oysters in mesh bags placed intertidally on the river bottom (Adams et al. 1991, Moroney 1997).

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POWER TO THE OYSTER: DO SPIONID-INDUCED SHELL BLISTERS AFFECT CONDITION IN SUBTIDAL OYSTERS?

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ABSTRACT Two static allometric condition indices and two static histological indices were assessed in determining whether oyster shell blistering induced by *Boccardia knoxi* infestations affected the health of infested oysters. Analysis of variance, Spearman's correlation analysis, and *a-posteriori* power analysis were used to assess the condition indices. The use of the dry weight condition index $CI_{\text{flesh shell}}$ and the shell volume condition index $CI_{\text{flesh CV}}$ were first validated after checking for confounding due to increased shell weight and loss of shell volume resulting from blistering. No significant differences in condition were detected between the sexes within the three subjective shell grades chosen to represent varying levels of shell blistering. Loss of condition was detected by three out of the four indices; the $CI_{\text{flesh shell}}$ and $CI_{\text{flesh CV}}$ both detected significant differences, but did not have the required level of power to reject the null hypothesis of no effect. However, for heavily blistered oysters the highly significant reduction in oocyte size (CI_{oocyte}) supported rejection of the null hypothesis. The loss of condition was considered insignificant in terms of subtidal oysters production, but demonstrates the parasitic effect spionids can have even under ideal growing conditions. The only index not detecting an effect, $CI_{\text{gonad area}}$ had the lowest power (<3%), illustrating the importance of performing *a-posteriori* power analysis. The results of the analysis of variance (ANOVA), Spearman's rank nonparametric correlation coefficients (SCC), and power analysis all indicated the $CI_{\text{flesh shell}}$ was the most sensitive of the four indices compared. The negative effects of shell blistering induced as a result of *B. knoxi* infestations rendered this species a parasite of Pacific oysters in Admiralty Bay.

KEY WORDS: Spionid polychaete, Spionidae, *Boccardia*, *Polydora*, Pacific oyster, *Crassostrea gigas*, shell blisters, aquaculture, subtidal, power analysis

INTRODUCTION

The effects spionid polychaete worm infestations can have on oysters have been confusingly portrayed in the literature. Some studies have detected a parasitic effect resulting in decreases in condition due to infestations (Wargo and Ford 1993, Lunz 1941) whereas others imply commensalism with no significant effect (Stephen 1978, Medcof 1946) or even greater condition (Schleyer 1991). Spionid worms can infest oysters by two means: boring through the shell structure from the external surface using metabolic acids (Haigler 1969), or entering the mantle cavity of the oyster as larvae and thus settling within the shell (Handley and Bergquist 1997, Skeel 1977, Whitelegge 1890). The latter results almost immediately in oysters secreting a blister of either conchiolin or shell material to wall off the intruder from the shell. In response to the former, shell blistering can result if the worms bore through the inside shell surface and penetrate the mantle cavity. Loss of condition is expected in oysters secreting shell blisters; as the oysters have to expend energy making the blisters (Blake et al. 1996), they lose internal shell volume, and the blisters' irregular shape may interfere with the feeding currents within the mantle cavity (Korringa 1951). The presence of blisters inside the mantle cavity also requires that the oysters increase shell deposition to regain internal shell volume and shape.

The cause of some of the confusion over the etiological status of spionids may be due to a combination of inappropriate methods used to detect spionid impacts and low statistical power when a null result is concluded. The relationship between environmental variables and the apparent "health" of oysters has been investigated using ecophysiological indices to summarize the physiological activity of the animals (Lucas and Beninger 1985). The most

popular methods used to measure physiological effects of spionid infestations in bivalves have been "static" condition indices which determine the health of the host at a single point in time using shell volume as a parameter (Wargo and Ford 1993, Lunz 1941). These indices may, however, be inappropriate if confounded by the loss of shell volume and increased shell deposition resulting from infestations (Handley and Bergquist 1997).

In New Zealand, shell blisters in Pacific oysters have been attributed to a number of spionid species, especially *Polydora websteri* in intertidal cultivations in the North Island (Handley and Bergquist 1997), and *Boccardia knoxi* infesting South Island subtidal cultivations (Handley 1995, 1997). In these studies a dry weight condition index recommended by Lucas & Beninger (1985) detected a biologically insignificant correlation with loss of condition in infested oysters in intertidal cultivations (Handley and Bergquist 1997), but no adverse impacts on condition in subtidal oyster populations (Handley 1997).

Condition indices relate the tissue weight of the animal to some estimate of shell size, with reductions in condition implying consumption of stored energy reserves (Newell 1985). The primary energy reserve stored by marine bivalves is the metabolite glycogen (Barber et al. 1988) which is responsible for the creamy condition and sweet taste of "fat" oysters (Beaumont and Fairbrother 1991). The gametogenic cycle of oysters is influenced strongly by the condition of oysters, with glycogen being used up during gametogenesis (Perdue and Erickson 1984, Mason and Nell 1995). One of the most accurate techniques reported to assess the metabolic costs of parasitism in oysters is the "gamete volume fraction" derived histologically from the ratio of the gonad to visceral area (Mori 1979, Perdue et al. 1981, Newell and Barber 1988). More recent histological techniques have been developed to compare the size of gametes to quantify any effect on gamete production (Barber et al. 1988). To quantify the true effects of a parasite,

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host individuals with infestation ranging from nil to severe must be sampled from within the same population (Newell and Barber 1988).

The aim of this study was to test the null hypothesis (H_0) that spionid-induced shell blistering did not affect the condition of subtidal Pacific oysters. This was done by using two static allometric condition indices and two static histological condition indices. The validity of the condition indices in terms of confounding were tested, and power and correlation analyses were used to compare the sensitivity of the condition indices.

METHODS

Experiment

Oysters were collected from the experimental oyster growing site "B" in Admiralty Bay, Marlborough Sounds (Handley 1997). One hundred and twenty intact oysters of a similar size were selected from a population of oysters grown on plastic sticks after they had been separated and scrubbed clean of fouling organisms. These oysters had previously been shown to contain a proportion of blistered oysters and were sampled on the 2 December 1995 which was the time during which *B. knoxi* had been previously recorded to induce shell blistering as gonad condition increased (Handley 1997).

Analysis

After cleaning, each oyster's shell volume was individually measured by displacement before being labelled with rubber bands and plastic tags (Roper et al. 1991, Medcof and Needler 1941). The oysters were carefully opened with a sharp knife and separated into three subjective categories: "0" containing no shell blisters; "1" containing 0–50% of the internal shell covered by blisters, and "2" containing <50% of the internal shell blistered. The oysters were then sexed by removal of gametes by a small scalpel incision of the gonad at the base of the adductor muscle. Eight oysters from each sex were then selected to best represent each category. The oyster meats were individually removed and weighed wet, before and after a standard transverse section had been removed from behind the labial palps (Wilson et al. 1993, Perdue et al. 1981). The gonad sections were fixed in Davidson's solution for 24 h and then transferred to 70% ethanol in preparation for histological sectioning and staining (Barber et al. 1988). The remaining meat fractions and shells were dried to a constant weight at 80°C (Roper et al. 1990) after the shucked shell displacement volumes had been measured.

Static Condition Indices

The dry weight ($CI_{\text{flesh:shell}}$) and shell cavity ($CI_{\text{flesh:cv}}$) condition indices (Roper et al. 1991) were derived from the above measurements after the whole dry meat weights had been determined from the dry meat weight fraction, given the assumption that the two wet weight fractions of oyster had the same water content when sectioned:

- (1) $CI_{\text{flesh:shell}} = \text{dry meat weight (mg)/dry shell weight (g)}$
- (2) $CI_{\text{flesh:cv}} = \text{dry meat weight (mg)/shell cavity volume (ml)}$

Histology

Histological sections 4–5 μm thick were obtained with a Jung 1130 rotary microtome and stained with a standard Ehrlich's he-

matoxylin and counterstained with Eosin (cf. Myers Hematoxylin; Barber 1996, Perdue et al. 1981).

Histological Indices and Image Analysis

The gamete volume fraction or "relative fecundity" ($CI_{\text{gonad:area}}$) and oocyte areas (CI_{oocyte}) were determined from the histological sections using OPTIMAS video image analysis software viewed and calibrated through a dissecting microscope and video camera (Barber 1996, Lowe et al. 1982, Perdue et al. 1981, and Barber et al. 1988). The area of 50 oocytes was measured using OPTIMAS:

- (1) $CI_{\text{gonad:area}} = \text{area of gonad (mm}^2\text{)/area of entire visceral mass (mm}^2\text{)}$
- (2) $CI_{\text{oocyte}} = \text{cross sectional area of 50 female gametes (}\mu\text{m}^2\text{)}$

Statistics

Fixed factor analysis of variance (ANOVA, PROC GLM; SAS 1992) was used to test the null hypothesis (H_0): spionid-induced shell blistering had no impact on oyster condition as measured for the balanced data for the $CI_{\text{flesh:shell}}$, $CI_{\text{flesh:cv}}$, and $CI_{\text{gonad:area}}$. Mixed model ANOVA was used to test the same H_0 for the balanced data for CI_{oocyte} using the mean squares error of the nested replicate interaction term to test significance between shell grades. The data sets were first \log_{10} transformed as they were growth related (LaBarbera 1989) and checked for normality and homogeneity of variances before ANOVA procedures. Spearman's rank nonparametric correlation coefficients (SCC, SAS; PROC CORR) were also produced to test the H_0 , and *a-posteriori* power analysis was performed for each ANOVA to determine the power of not committing a "Type II" error or accepting a false H_0 (Searcy-Bernal 1994, Koele 1982).

RESULTS

Oysters grown in the vicinity of this site have a history of spionid infestations with 60% containing shell blisters due to infestations dominated by *Boccardia knoxi* (Handley 1995, 1997). Visually there appeared to be no differences in the condition of the oysters, with all oysters in a "fat" condition. Histologically, all gonads were approaching "stage 3", the females having teardrop-shaped eggs and follicular channels or "curtains" evident, the male follicles being dark (basophilic) with sperm heads, and the center consisting of sperm tails which were paler staining (Wilson et al. 1993, Dinamani 1974).

Although a trend was evident in the $CI_{\text{flesh:shell}}$ and $CI_{\text{flesh:cv}}$ data suggesting that females had greater condition index values than males, the 95% confidence intervals showed there was no significant difference between sexes (Fig. 1) and the mixed model ANOVAs failed to detect a significant difference between sexes for each of the condition indices (Table 1A–F). These data were subsequently lumped within shell grades and reanalyzed. ANOVA comparing means between shell grades using the $CI_{\text{flesh:shell}}$ and $CI_{\text{flesh:cv}}$ both detected a loss of condition due to shell blistering between shell grades (Table 2, Fig. 2). These indices were not confounded, with no significant differences detected between dry shell weights or shell volumes between shell grades (Table 1B & D, Table 2). The dry meat weights were, however, significantly different between grades across both sexes (Table 1A, Table 2). Decreasing shell quality was significantly correlated with a decrease in condition measured by the $CI_{\text{flesh:shell}}$ but not by the

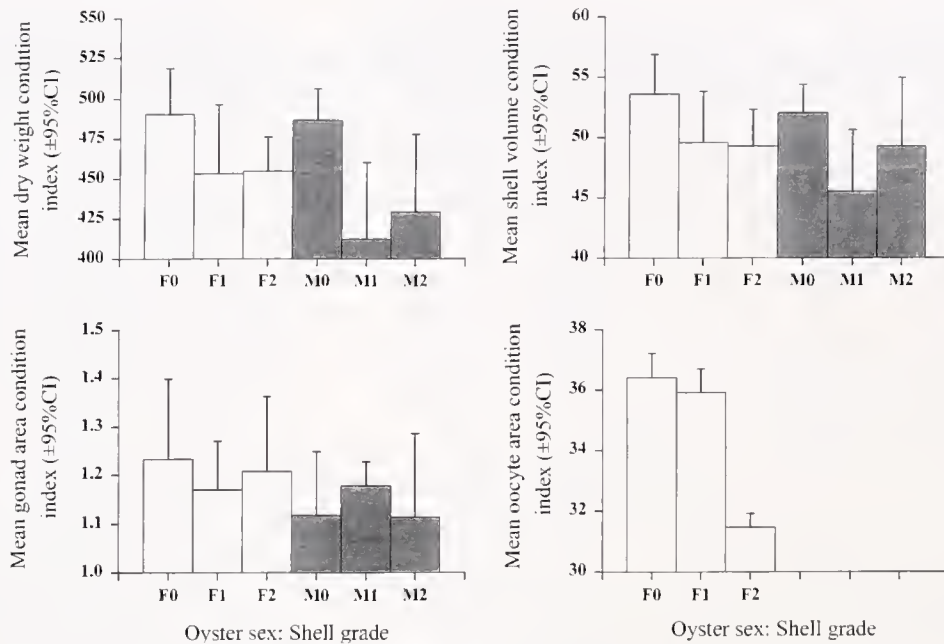


Figure 1. Condition index values for oysters separated by sex: F, females (clear bars); M, males (grey bars); shell blister grades: 0, 1, 2.

$CI_{flesh:cv}$ (Table 2). The statistical power of the $CI_{flesh:shell}$ test for rejecting the H_0 was also greater than for the latter $CI_{flesh:cv}$.

The fixed model ANOVA and SCC for $CI_{gonad:area}$ failed to detect a significant impact or correlation of shell blistering impacts, with this index having the lowest power (<3%) of all three indices, with the same degrees of freedom. The mixed model ANOVA and SCC for CI_{oocyte} , however, detected a significant negative impact and correlation of decreasing oocyte size with increased shell blistering (Table 2). The oysters with greater than 50% of the shell affected by shell blisters produced significantly smaller oocytes (Fig. 1). A significant difference was also detected between oysters nested within shell grades, but this was not surprising given the high degrees of freedom of this test (Table 1).

DISCUSSION

The two static condition indices $CI_{flesh:shell}$ and $CI_{flesh:cv}$ are ecophysiological indices that summarize the apparent "health" or physiological activity of cultured bivalves (Lucas and Beninger 1985). In this study both these indices detected significant impacts of shell blistering on oyster condition. It was previously thought that the presence of shell blisters within the mantle cavity of oysters may increase either the shell dry weights or the shell cavity volumes, thus confounding these indices (Handley and Bergquist 1997). Two or more effects are said to be "confounded" in an experiment if it is impossible to separate the effects after the statistical analysis has been performed (Ostle and Mensing 1975). Confounding would occur in the case of the $CI_{flesh:shell}$ and $CI_{flesh:cv}$ if either the shell dry weights or shell cavity volumes were affected significantly by shell blistering, thus allowing for more than one possible null hypothesis to be tested. By determining no significant effect of shell blistering against the denominator values of $CI_{flesh:shell}$ and $CI_{flesh:cv}$, the results of these two indices were validated.

In this study, power analysis was used to compare the sensitivity of the static condition indices given the same degrees of freedom. "Statistical power is the probability of not committing a

Type II error, or accepting a false null hypothesis" (Searcy-Bernal 1994). In basic research power analysis, the cost of Type I error most often greatly exceeds the cost of Type II error (Toft and Shea 1983). Power analysis for the simple one-factor ANOVAs after the data had been lumped for $CI_{flesh:shell}$ and $CI_{flesh:cv}$ did not reach the required level of precision accepted by many authors (80%, Searcy-Bernal 1994). However, given the highly significant negative SCC and the rejection of H_0 by the ANOVA tests for $CI_{flesh:shell}$, $CI_{flesh:cv}$, and CI_{oocyte} , all results support acceptance of an impact on condition. Further, the results of the CI_{oocyte} showed a highly significant decrease in size of the oocytes from oysters with greater than 50% of their shell blistered. The power of the ANOVA test for CI_{oocyte} was not calculated, as power analysis involving mixed model interaction terms is not advised as a number of severe restrictions have to be made on mixed model covariance matrices; even then it is only possible to derive the distribution under the null hypothesis. When in some instances power can be calculated for mixed models, this power value must be understood to represent the lower bound of the power against a class of alternatives, and not the exact power against a specific alternative (Koele 1982). The results for the CI_{oocyte} were, however, highly significant and surpassed the other indices at the 95% confidence level. Given the same degrees of freedom, the $CI_{flesh:shell}$ had greater statistical power over the $CI_{flesh:cv}$, and the significant correlations detected by $CI_{flesh:cv}$ further support the conclusions of Lucas and Beninger (1985) that the $CI_{flesh:shell}$ is a more sensitive indicator of condition than $CI_{flesh:cv}$ in oysters. The $CI_{flesh:cv}$ has also been criticized as it is relatively insensitive to low signal-to-noise ratio and therefore significant changes to the index are only likely to be evident after long-term exposures (Widdows 1985).

Power analysis can also be used to determine the power of not detecting an impact when one was present (Searcy-Bernal 1994). The danger of this was highlighted by the low power of the $CI_{gonad:area}$ test (<3%). This index has been described as one of the most accurate techniques for assessing parasitism (Newell and Barber 1988), however, in this study, the power of the test was too

TABLE 1.

Analysis of variance for (A) oyster dry meat weights, (B) oyster dry shell weights, (C) oyster dry weight condition index, (D) oyster shell volume, (E) oyster volume condition index, (F) oyster gonad/visceral mass condition index, and (G) oyster oocyte area condition index December 1995.

A						
Test of hypotheses for fixed model ANOVA:						
Dependent variable: Dry meat weights						
Source	DF	Type III SS	MS	F	Pr > F	
Shell Grade	2	0.138	0.069	4.89	*	
Sex	1	0.046	0.046	3.23	ns	
ShellGrade*Sex	2	0.005	0.002	0.16	ns	
B						
Test of hypotheses for fixed model ANOVA:						
Dependent variable: Dry shell weights						
Source	DF	Type III SS	MS	F	Pr > F	
Shell Grade	2	0.023	0.011	1.11	ns	
Sex	1	0.019	0.019	1.85	ns	
ShellGrade*Sex	2	0.015	0.008	0.75	ns	
C						
Test of hypotheses for fixed model ANOVA:						
Dependent variable: CI _{flesh shell}						
Source	DF	Type III SS	MS	F	Pr > F	
ShellGrade	2	28424.168	14212.084	5.05	*	
Sex	1	6041.096	6041.0957	2.36	ns	
ShellGrade*Sex	2	2774.957	1387.478	0.49	ns	
D						
Test of hypotheses for fixed model ANOVA:						
Dependent variable: Shell volume						
Source	DF	Type III SS	MS	F	Pr > F	
ShellGrade	2	0.071	0.036	2.82	ns	
Sex	1	0.045	0.045	3.60	ns	
ShellGrade*Sex	2	0.069	0.034	2.72	ns	
E						
Test of hypotheses for fixed model ANOVA:						
Dependent variable: CI _{flesh cv}						
Source	DF	Type III SS	MS	F	Pr>F	
ShellGrade	2	232.112	116.056	3.30	*	
Sex	1	42.803	42.803	1.22	ns	
ShellGrade*Sex	2	33.689	16.844	0.48	ns	
F						
Test of hypotheses for fixed model ANOVA:						
Dependent variable: CI _{gonad area}						
Source	DF	Type III SS	MS	F	Pr>F	
ShellGrade	2	0.002	0.001	0.03	ns	
Sex	1	0.053	0.053	1.38	ns	
ShellGrade*Sex	2	0.035	0.018	0.45	ns	
G						
Test of hypotheses for mixed model ANOVA:						
Dependent variable: CI _{oocyte}						
Source	DF	Denominator	Denominator	MS	F	Pr > F
		Type III SS	DF			
ShellGrade	2	0.234	21	0.052	4.545	**
Oyster (ShellGrade)	21	0.052	576	0.006	9.042	***

DF, degrees of freedom; SS, sums of squares; MS, mean squares; F, f statistic; Pr > F; ***Pr < 0.001, **Pr < 0.01, *Pr < 0.05.

low to determine whether shell blistering affected relative fecundity without increasing the sample size. As a loss in condition was detected by the other indices, the results of the CI_{gonad area} test provided a good example of the value of calculating the power of statistical tests before accepting the null hypothesis, given that the

four condition indices used on the same oysters in this study produced different results with highly variable statistical power, it is not surprising there is confusion in the literature as to the etiological status of spionids around the world.

Despite a measurable decrease in condition detected in this

TABLE 2.

Analysis of variance, Spearman's correlation coefficients (SCC) and power values for oyster dry meat weights, oyster dry shell weights, oyster dry weight condition index, oyster shell volume, oyster volume condition index, oyster gonad/visceral mass condition index, and oyster oocyte area condition index, December 1995.

Test of hypotheses for fixed model ANOVA and SCC:									
Source: Shell grade									
Dependent variable	DF	Type III SS	MS	F	Pr > F	'Power' of ANOVA	SCC	Pr > R	
Dry meat weights	2	0.138	0.069	4.83	**	73.3%	-0.293	*	
Dry shell weights	2	0.023	0.011	1.10	ns	22.8%	-0.088	ns	
CI _{flesh:shell}	2	28424.168	14212.084	5.01	**	75.4%	-0.390	***	
Shell volume	2	0.071	0.036	2.49	ns	25.3%	-0.371	***	
CI _{flesh:cv}	2	232.113	116.056	3.36	*	57.8%	-0.228	ns	
CI _{gonad:area}	2	0.002	0.001	0.03	ns	2.8%	-0.061	ns	
Test of hypotheses for mixed model ANOVA and SCC:									
Source: Shell grade									
Dependent variable	Denominator DF	Denominator Type III SS	DF	MS	F	Pr > F	'Power' of ANOVA	SCC	Pr > R
CI _{oocyte}	2	0.234	21	0.051	4.55	**	—	-0.284	***

DF: degrees of freedom, SS: sums of squares, MS: Mean squares, F: f statistic, Pr > F; ***Pr < 0.001, **Pr < 0.01, *Pr < 0.05.

study, the slight loss of condition is unlikely to be biologically significant in terms of survival of the oysters in the subtidal environment where ideal conditions for growth are found. The infested oysters seemed capable of easily sustaining the loss of internal shell volume, irregular shell shape, and the increased shell deposition associated with shell blistering. Likewise, in terms of aquaculture production, the impacts of the spionid infestations on oyster meat production was negligible if one ignores the loss in value to the half-shell trade due to the presence of the blisters. In ecological terms, however, the heavily blistered oysters produced significantly smaller oocytes, thus infestations may retard oocyte development, spawning, or have implications for larval survival. In a

study of the effects of the *Haplosporidium* parasite 'MSX' on *C. virginica*, "relative fecundity" (CI_{gonad:area}) was significantly reduced but no significant differences were detected in the size of the mature oocytes (Barber et al. 1988). Stress-induced reductions in oocyte size have been measured in the Blue mussel *Mytilus edulis* and shown to decrease the nutritive reserves, thus significantly reducing the viability of the larvae produced (Bayne 1975, Bayne et al. 1978). Recruitment was therefore reduced even though the total number of gametes did not differ between stressed and non-stressed individuals (Newell and Barber 1988). These subtle effects of parasitism may be more common than is generally thought and may have severe consequences for bivalves if they are stressed

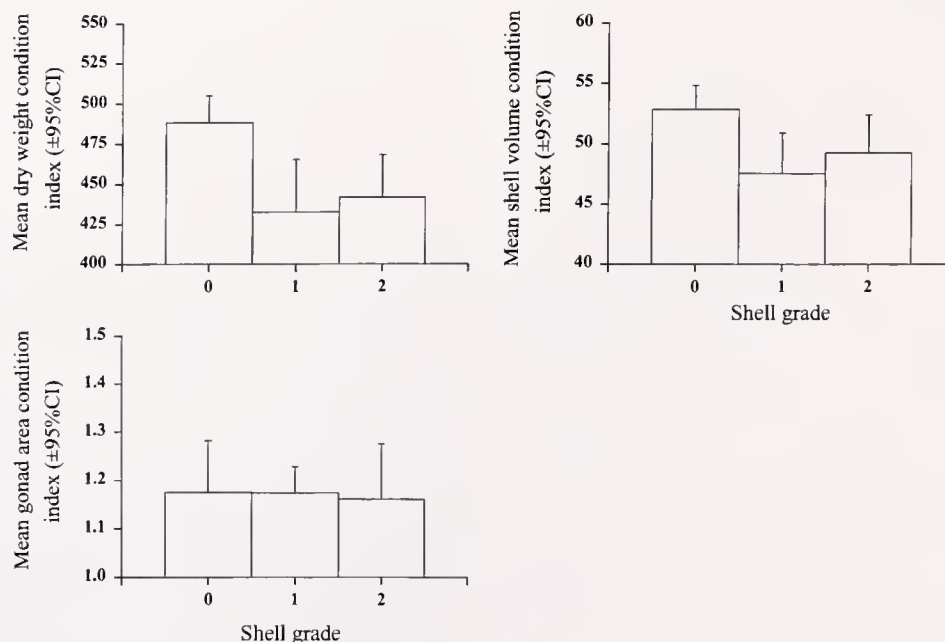


Figure 2. Condition index values for oysters for combined sexes; shell blister grades: 0, 1, 2.

due to adverse environmental change, given the intuitively attractive concept that stress lowers resistance, rendering individuals more prone to parasitism (Newell and Barber 1988). Thus, spionid-induced impacts may not be trivial for all conditions, for example, if the oysters are in a stressed state or in poor condition because of overcrowding, limiting food availability. Shell blistering under these circumstances could produce commercially and biologically significant effects. As gonad development is linked to reductions in nutritive reserves of glycogen (Perdue and Erickson 1984), the state of gonad development would play an important role in the response of the oysters to the intrusions of spionid polychaetes within their shells. As there are reports of spionid infestations causing mortality in oyster populations (Nelson and Stauber 1940, Whitelegge 1890, Wisely et al. 1979), further investigations are needed into their effects over varying stages of the gametogenic cycle and under different environmental stresses. These studies could help elucidate the stress thresholds that render the oysters susceptible to shell blistering which result from spionid infestations.

Spionid polychaetes do not derive a nutritional benefit from their host; rather they are in loose association with their host (Rohde 1982, 1993). Parasitism has been defined as "a close association between two organisms, one of which, the parasite, depends on the other, the host, deriving some benefit from it" (Rohde 1982). Spionids species that have no obvious effect on the host can be classified as "latent parasites" which have no obvious effects on the host (Rohde 1993). For example, the *Boccardia* species found infesting the external shells of intertidal oysters in

the Mahurangi Harbour had no apparent effect on the oysters (Handley and Bergquist 1997). Some researchers have implied commensalism by spionids whereby the spionids utilize food supplied in the external or internal environment of the host (Thomson 1954). If the association damages the host, then the commensal becomes a parasite (Rohde 1982). The results of this, and previous studies detecting negative impacts of spionid worms on oysters, render them parasites, for example, if they induce the formation of shell blisters either during settlement or during the process of burrowing within the shell (Odening 1976). Further debate about the biological significance of spionid infestations will continue until experiments integrating a range of gametogenic conditions and environmental stresses are carried out to determine the thresholds of stress induced by these parasites. Such studies could have important practical applications in aquaculture pest management.

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OYSTER REEF BROODSTOCK ENHANCEMENT IN THE GREAT WICOMICO RIVER, VIRGINIA

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ABSTRACT The Great Wicomico River is a small, trap-type estuary on the western shore of the Chesapeake Bay that once supported substantial oyster populations. These populations were essentially eliminated by the combined effects of Tropical Storm Agnes in 1972, and subsequent disease mortalities related to *Perkinsus marinus* and *Haplosporidium nelsoni*. Oyster broodstock enhancement was initiated in June 1996 by the construction of a three-dimensional intertidal reef with oyster shell, followed by the "seeding," in December 1996, of that reef with high densities of large oysters from disease-challenged populations in Pocomoke and Tangier Sound. Calculations of estimated fecundity of the reef population suggest that oyster egg production from this source is within an order of magnitude of total egg production in the Great Wicomico River prior to Tropical Storm Agnes. Field studies in 1997 indicate spawning by reef oysters from July through September. *P. marinus* prevalence increased from 32% in June to 100% in July, whereas intensity increased from June to September; *H. nelsoni* was absent. Plankton tows recorded oyster larval concentrations as high of $37,362 \pm 4,380 \text{ m}^{-3}$ on June 23. Such values are orders of magnitude higher than those typically recorded in Virginia subestuaries of the Chesapeake Bay in the past three decades, and lend support to a premise that aggregating large oysters may increase fertilization efficiency. Drifter studies suggest strong local retention of larvae, a suggestion reinforced by marked increases in local oyster spatfall on both shellstrung collectors and bottom substrate compared with years prior to 1997. In locations where local circulation promotes larval retention, the combination of reef construction with broodstock enhancement may provide an accelerated method for oyster population restoration.

KEY WORDS: Oyster, *Crassostrea virginica*, Great Wicomico River, reefs, fecundity, larvae, oyster settlement

INTRODUCTION

The Eastern oyster, *Crassostrea virginica* (Gmelin), plays an important ecological role in the Chesapeake Bay and its tributaries as well as being the focus of a substantial commercial fishery. Oyster reefs developed in recent geological time as the current Chesapeake Bay was inundated by rising sea level. By early Colonial times, oyster reefs had become significant geological and biological features of the Bay. Intensive exploitation since Colonial times, combined with more recent impacts of two protistan parasites, *Perkinsus marinus* ("Dermo") and *Haplosporidium nelsoni* ("MSX"), have led to the degradation of these reefs such that only two-dimensional "footprints" of these former reefs remain. Today, these "footprints" maintain drastically reduced oyster populations. The Virginia Marine Resources Commission (VMRC) supports an extensive replenishment program throughout most of its portion of the Bay. Traditional replenishment programs have focused on spreading thin veneers of shell substrate for larval settlement over coastal and estuarine bottoms. The purpose of this practice is to provide a suitable substrate for settlement at minimum cost. Ideally, the end product is the retrieval of seed or market-size oysters from these shell "plants"; however, these thin, two-dimensional carpets bear little resemblance to the intricate, three-dimensional reefs that once supported a large oyster population.

More recent replenishment programs have focused on the construction of three-dimensional reefs that resemble more closely what was found in Colonial times. Since 1993, reefs have been constructed in the Piankatank, Great Wicomico, Coan, Yeocomico, and James Rivers in the Virginia portion of the Chesapeake Bay, and Lynnhaven Bay on the southern side of the Bay mouth. These reefs are built on the "footprints" of former reefs and consist of several mounds of shell that protrude out of the water at low tide (e.g., Bartol and Mann, 1997). Reef communities have been allowed to mature naturally with no addition of brood-

stock oysters to the reef based on the premise that oysters would recruit to the reef from the plankton, and because there was no resident population of disease-infected oysters, would develop as a predominantly disease-free population. This was not found to be the case on an artificial reef built in the Piankatank River, Virginia (Mann et al. 1996, Mann and Wesson 1996). Endemic diseases did become established in the reef populations; however, the vertical relief of the reefs enhanced growth to such an extent that the oysters grew larger and faster than on adjacent "flat" oyster reefs. From 1993 to 1996 oyster populations on the Piankatank River reef developed to densities of 50–70 oysters m^{-2} (Mann and Wesson unpublished data). This compares with densities of 200–350 m^{-2} on the most commercially productive reefs (flat) in the James River system (Mann and Wesson unpublished data, Mann and Evans 1998). The disparity in these values suggests that development of very dense and stable oyster communities on constructed reefs is a long-term event that is delayed in regions that suffer poor natural recruitment, and may be accelerated with an initial stocking of broodstock.

The current study describes the impact on local plankton communities and oyster settlement of an artificial reef, constructed in 1996–1997 in the Great Wicomico River (Fig. 1) that was initially "seeded" with reproductively capable oyster populations. Specifically, the study sought to determine temporal spawning patterns of "seeded" oysters, estimate the fecundity and larval production from oysters on the reef, and examine subsequent larval abundance, distribution, and settlement in relation to local circulation patterns.

METHODS AND MATERIALS

Data Collected as Part of Long-Term Monitoring

In order to describe the ecological impact of the reef, it is necessary to present 1997 data in the context of a brief historical

description of oyster populations. Original surveys of the limits of oyster distribution in the Great Wicomico River were provided by Baylor (1896) and subsequently revised by Haven et al. (1981). Temporal (intra- and inter-annual) description of oyster settlement (spatfall), population density, and demographics are available from continuing Virginia Institute of Marine Science (VIMS) stock monitoring programs.

The spatfall survey has been completed annually from 1965 to the present. The collectors used to monitor spatfall were oyster-shellstrings, which consist of 12 oyster shells of similar size (about 76 mm, max. dimension) drilled through the center and strung (inside of shell down) on heavy gauge wire. Shellstrings were hung 0.5 m off the bottom at each station. Up to 16 stations have been used at various times throughout the history of the spatfall surveys; however, for consistency between years, this study reports only the six stations (see Fig. 1) that have been used yearly since 1965. Shellstrings were replaced after a 1-week exposure (with occasional deviations) from June through September, and the number of spat that attached to the smooth underside of the middle 10 shells were counted with the aid of a dissecting microscope.

The fall dredge survey provides information about spatfall and recruitment, summer mortality, and inter-annual changes in abundance of seed and market-size oysters. This survey has been completed yearly from 1971 to the present, excluding 1974–1976. Figure 1 shows the geographical locations of the bars sampled in the Great Wicomico River during this time. As with the shellstring data, only the most consistently sampled stations were used in the analysis. Three stations (Fleet Point, Whaley East, and Haynie Point) have been sampled since 1986. Analysis was limited to

these three stations. Three to four 0.5 bushel samples of bottom material were taken at each bar using a 24-inch dredge having 4-inch teeth. For each sample the following were determined: number of market-size oysters (>76 mm, max. dimension), number of small oysters (below market size and yearlings), and the number of spat (young of the year oysters). In the case where only 0.5 bushels were counted, they were standardized to one bushel by doubling the counts. In the fall of 1995 and 1997, a collaborative survey effort between VMRC and VIMS resulted in a formal stock assessment on the oysters in the Great Wicomico River using patent tongs (Chai et al. 1992). The five oyster reefs that were sampled in the Great Wicomico River in 1995 and 1997 are shown in Figure 1. For each reef a uniform grid was generated over a current reef boundary map. Each grid location had a reference that could be located electronically by LORAN from the research vessel. Grid references were assigned a sampling order from a random number table to generate a randomized sampling grid. Samples were collected using hydraulic patent tongs with an opening of 1 m². All of the retained material was washed, and counts of live oysters as spat (young of the year), small oysters (<76 mm, max. dimension), and market oysters (>76 mm, max. dimension) were taken. Adequacy of sampling was assured using guidelines of Bros and Cowell (1987), as described in Mann and Evans (1998).

Estimation of Historical Egg Production, Fertilization and Embryo Production

To place in context the impact of adding broodstock, estimates of historical egg production when adult oysters were still abundant

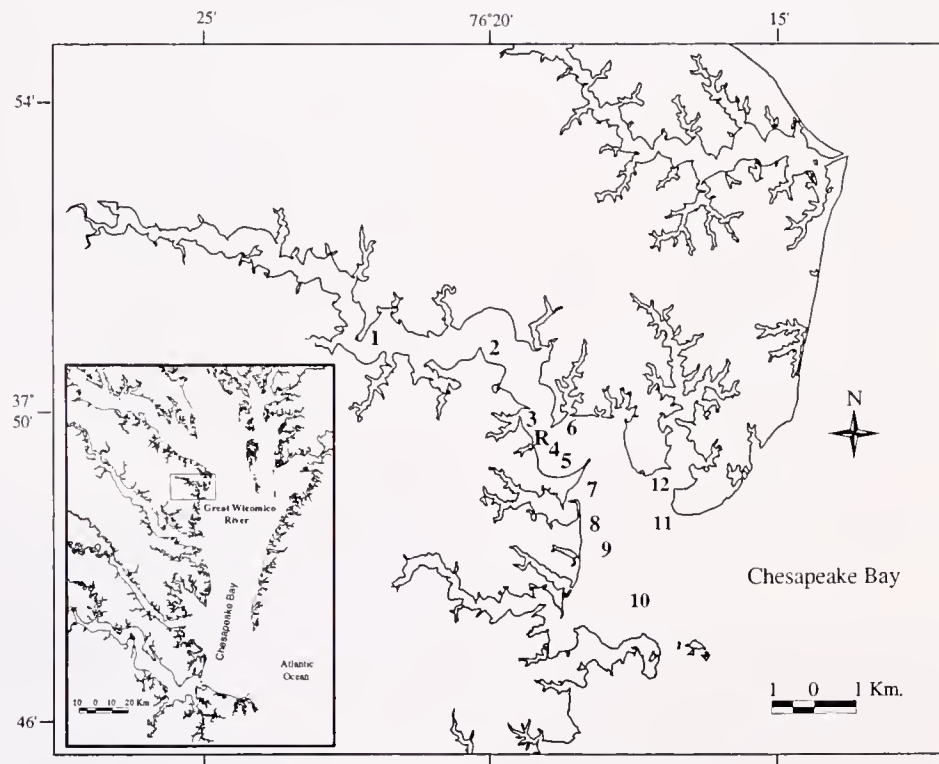


Figure 1. Map of the Great Wicomico River showing the locations of various stations used throughout this study (1–12) and the location of the artificial reef (R). Shellstring stations are represented by sites 1, 3, 6–7, 9, and 11, fall dredge stations are represented by sites 6, 7, and 9, patent tong stations are represented by sites 4–5, 7, and 10–11. Egg production was calculated using the area of shell bottom covering sites 2–12. Inset shows the location of the Great Wicomico River within the Chesapeake Bay system. The numerical key identifying stations corresponds to that used throughout the current text. 1: Glebe Point, 2: Rogue Point, 3: Hudnall's, 4: Shell Bar, 5: Sandy Point, 6: Haynie Point, 7: Cranes Creek, 8: Whaley West, 9: Whaley East, 10: Dameron Marsh/Ingram, 11: Fleet Point, 12: Cockrell Creek.

in the river are required. Quantitative historical stock assessment data for the Great Wicomico are lacking, but there are current data for extant reefs in the James River in similar salinity regimes which have similar qualitative (based on dredge survey data) population demographics. A combination of revised Baylor survey data (Haven et al. 1981) of reef area in the Great Wicomico (Fig. 1) and current James River quantitative stock assessment data (Mann and Wesson unpublished, Mann and Evans 1998) were used to estimate historical oyster demographics in the Great Wicomico (see Table 1). Since salinity plays a role in reproductive success, it was necessary for the reefs being compared to have similar salinity regimes. Reefs in three salinity regimes (8.5, 10.5, and 13.5 ppt, see Mann and Evans 1998) in the James River were used in the calculation. Egg production per unit area for each reef in the James River, based on the appropriate size frequency distribution, was estimated by methods described in detail in Mann and Evans (1998) using size-specific fecundity taken from Thompson et al. (1996), parity in sex ratio as suggested by Cox and Mann (1992), and density-dependent fertilization efficiency as described by Levitan (1991).

1997 Field Studies

The location selected for the study was Shell Bar Reef in the Great Wicomico River, Virginia (location R in Fig. 1). The reef was constructed in June of 1996 by deploying old oyster shells from a barge with a crane into a series of intertidal structures approximately 215 m long and 18 m wide. Broodstock oysters from the Tangier and Pocomoke Sound regions were planted on the reef in December of 1996. Oyster standing stock and density was obtained from VMRC records (Olsen and Wesson 1997). According to these records, 2,281 bushels of oysters were planted on the 3,900 m² reef in December of 1996. Estimating 500 oysters per bushel (Wesson, personal communication), density of broodstock oysters on the reef was approximately 300 m⁻². Oysters surviving as sparsely distributed individuals in many regions of the

Bay are continually exposed to intense disease challenge and selection pressure. Consequently, they would be expected to have higher resistance to disease than low salinity populations where intermittent disease pressure fails to eradicate genetically susceptible individuals, which then continue to breed with more resistant individuals and thus fail to promote the process of developing uniformly high resistance. Tangier and Pocomoke Sounds are locations where higher salinities (25–30 ppt) occur, but oyster densities are low (<1 m⁻²), thus failing to maximize the fertilization efficiency. The intent of aggregating the few remaining oysters from disease endemic areas was to increase fertilization efficiency of freely released gametes.

Field studies were conducted biweekly from the 23rd of June through the 22nd of September 1997 (total of 8 field days). This time frame was chosen based on the historical timing of spat settlement in the Great Wicomico River system. To obtain a description of tidal patterns of circulation and larval abundance in the system, all sampling was effected over one complete tidal cycle (approximately 12 h). Surface temperature for all field studies was measured near the reef (Station N1; in Fig. 2) throughout the duration of the study. Temperature and salinity at the surface and bottom of the water column were obtained at three sites (Fig. 2) starting on July 28th (dates of collection coincided with the circulation study). Bottom water was collected using a Niskin bottle. Temperature was measured with an alcohol thermometer and salinity was measured with a refractometer.

Oyster Reproductive Biology and Disease Status

Initial broodstock oyster size frequency on the reef was obtained by measuring 150 oysters collected with the aid of hand tongs. Total egg production on Shell Bar reef, after broodstock enhancement, was calculated using density and size frequency data, as described in detail in Mann and Evans (1998).

Temporal patterns of gametogenic development of the broodstock oysters on the reef was examined by collection, with hand

TABLE 1.

Estimates of historical egg production in the Great Wicomico River using demographics obtained from analogous reefs in the James River in 1993.

Reef #	Reef Area m ²	Egg Production 10 ⁶ m ⁻²	Total # of Eggs * 10 ¹²	Salinity to Estimate Fs	Fs	Ff	Corrected Production 10 ⁶ m ⁻²	Corrected Total # of Eggs * 10 ¹²
1	29355	1160	34	8.5	0.09	0.16	16.8	0.49
2	23475	565	13	10.8	0.51	0.13	38.5	0.9
3	3636	565	2	10.8	0.51	0.13	38.5	0.14
4	29462	565	17	10.8	0.51	0.13	38.5	1.1
5	33261	565	19	10.8	0.51	0.13	38.5	1.3
6	83452	136.2	11	13.5	1	0.04	4.9	0.41
7	137735	136.2	19	13.5	1	0.04	4.9	0.68
8	251573	136.2	34	13.5	1	0.04	4.9	1.2
9	82723	136.2	11	13.5	1	0.04	4.9	0.41
10	22047	136.2	3	13.5	1	0.04	4.9	0.11
11	71929	136.2	10	13.5	1	0.04	4.9	0.35
TOTAL			173					7.1

See Mann and Evans (1998) for explanation of calculations.

Reef area—taken from the Great Wicomico Baylor survey data (Haven et al., 1978).

Egg production—calculated from size-specific fecundity of oysters in the James River (average of all reefs in the James River with the same salinity).

Total # of eggs—reef area in the Great Wicomico multiplied by egg production (based on James River demographics).

Fs—correction for salinity, based on reefs sharing similar salinities in both rivers.

Ff—correction for fertilization efficiency, based on densities found in the James River (also used for egg production calculations).

Corrected production—egg production/m² corrected for salinity, fertilization efficiency, and disease (production * Fd * Fs * Ff).

Corrected total # of eggs—corrected egg production multiplied by reef area (in the Great Wicomico).

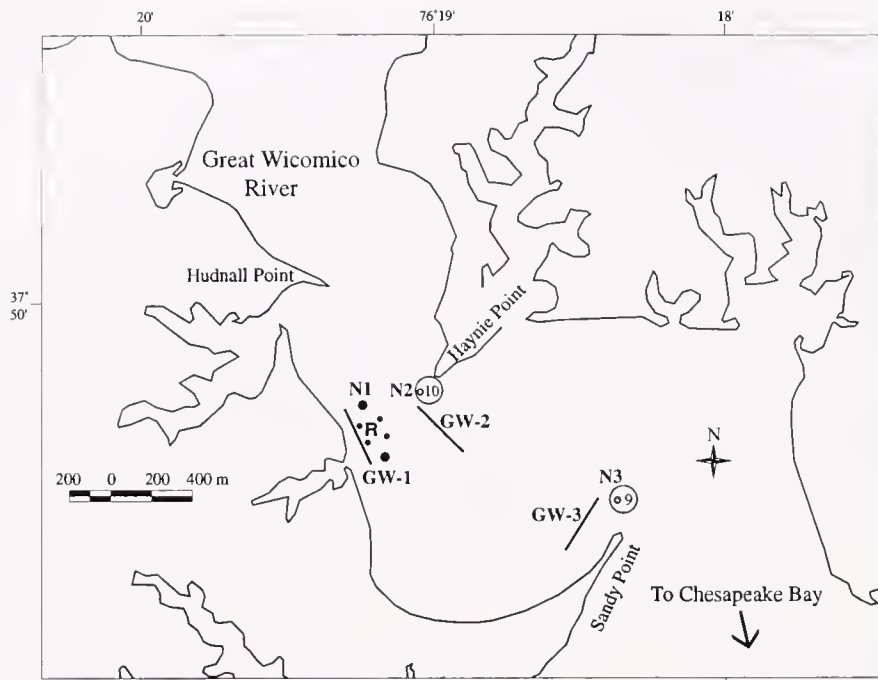


Figure 2. Location of zooplankton (GW 1–3) samples and water samples (N 1–3) taken in the Great Wicomico River. R denotes the location of the reef; 9 & 10 mark the main channel in the river.

tongs, of 25 oysters per sampling day for a total of 200 oysters. Sections of the gonad and visceral mass were removed and fixed in Bouin's solution. Following fixation, specimens were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Histological sections were cut at 7–10 μm , stained in Delafield's hematoxylin, and counterstained in eosin Y following the methodology of Humason (1962). Developmental stages were identified based on those originally described for *C. virginica* by Kennedy and Battle (1964) and for *C. gigas* by Mann (1979). Stages of gonadal development were defined as follows:

- (1) *Inactive*: No evidence of the presence of follicles peripheral to the digestive gland. Sex is essentially indeterminate.
- (2) *Early active*: **Male**. Many follicles filled primarily with spermatogonia and spermatocytes. No spermatozoa. **Female**. Eggs not well developed. A few nuclei in oocytes, but no nucleoli. Oocytes are still attached to the follicle wall.
- (3) *Late active*: **Male**. Follicles predominately filled with spermatids. Characteristic swirling pattern of spermatozoa with tails oriented toward the center beginning to be evident, but follicle is not completely filled. **Female**. Some free oocytes. Most have distinct nuclei, with fewer than 50% having distinct nucleoli.
- (4) *Ripe*: **Male**. Swirling of tails in the middle of the follicle. **Female**. Primarily free oocytes. Greater than 50% have a distinct nuclei and nucleoli. All of the oocytes are about the same size.
- (5) *Spawning or spent*: **Male**. Most follicles are empty or partially so. Some phagocytes present. **Female**. Granular looking eggs (amebocyte activity). Eggs of varying sizes that appear to be breaking down. Follicles are empty or partially so.

Monthly assays to determine *Perkinsus marinus* and *Haplosporidium nelsoni* (MSX) infections were effected using oysters collected for the reproductive development portion of the study. *Perkinsus* infection and prevalence were measured by Fluid Thioglycollate assay (Ray 1963). MSX infections were detected using paraffin histology, as in Bureson et al. (1988).

Plankton Studies

Plankton Studies

A series of 36 zooplankton samples were taken on each sampling day (three replicates per site, per tidal stage). Samples were collected at three stations in the river (Fig. 2). Plankton samples at GW-1 describe larval abundance near the reef, GW-2 describes abundance in the main channel of the river, and GW-3 describes abundance near the sand spit at Sandy Point, a feature that affects and effects some local retention in the system. Samples were collected using a 0.3 m diameter, 3:1 aspect ratio zooplankton net (Sea Gear Corporation, Melbourne, FL). The filtering surface consisted of an 80 μm Nytex mesh cone attached to a PVC collection bucket lined with 80 μm mesh. The net was attached to a metal ring and towed by a three-point bridal system attached to the ring. The net was towed 0.05–0.10 m below the water surface at approximately 1.5 m sec^{-1} for 3.25 min. The nets used were calibrated in a separate study following the same protocol (Harding and Mann, in review). Samples were taken over a full tidal cycle. All samples were immediately preserved in 95% ethanol.

Samples were split using a 0.5 L Folsom plankton splitter (Wilco Supply Company, Cass, MI). Final splits were filtered through a 400 μm Nytex mesh filter to remove large zooplankton that interfered with the counting. To ensure that no oyster larvae were lost in this process, samples were randomly chosen and counts were made before and after filtering. The difference between these counts was less than 1%. Non-enumerated splits, as well as the filtrate from the final splits, were archived. Counts of umbo stage oyster veligers (larvae) in each subsample were made

with the aid of a dissecting scope. To verify adequate mixing (i.e., a homogenous mixture of larvae within the sample), both halves of the final split were counted, and coefficients of variation (CV) were calculated following Van Guelpin et al. (1982). Acceptable CVs for invertebrate samples range from 5 to 20%. Counting error of the total abundance of organisms within a sample was kept to 10% or less by ensuring (when possible) that at least 100 veligers were counted from each subsample. Total number of larvae per sample was obtained by multiplying the number of veligers in the split by the split number. The number of larvae per m^3 was then obtained by dividing the total number per sample by the volume of water filtered. The mean volume of water filtered, was determined to be 1.054 m^3 in a separate net calibration study (Harding and Mann, in review).

Circulation Studies

Simple surface drogues (drifters) were constructed after the method of Davis et al. (1982) (Fig. 3). This design was used to ensure that the drifter was moved by the currents in the system with little input from the wind. The drifters were released at various sites around the reef and in the main channel of the river. The drifter locations were recorded approximately every hour using a hand held GPS system. The drifters were followed over one full

tidal cycle. In the event that a drifter ran aground, it was repositioned to another location, with exact location depending on the stage of the tide. Throughout the course of the sampling season, a total of 23 drifter paths were obtained on 5 separate days. Of the 23 drifter tracks obtained, 6 were discarded because of multiple lost GPS points and 3 were discarded because of excessive stoppage (they ran aground at least three times). This left a total of 14 drifter tracks to be analyzed. Drifter time and location information was loaded into the Geographical Information System/ArcView computer program in the Coastal Inventory Program at VIMS. The drifter paths were then plotted in ArcView software and mean current speeds were estimated for each series of drifter recordings. These were then compared with predicted tidal flow for Sandy Point in the Great Wicomico River system (Tides and Currents for Windows, version 2.2, Nautical Software Inc.).

RESULTS

Data Collected as Part of Long-Term Monitoring

Spatfall estimates obtained from the historical shellstring data are summarized in Table 2. From 1965 through 1971 spatfall in the Great Wicomico River was relatively high with mean weekly values (number of larval oysters physically adhering to the substrate) ranging from 1 to 494 spat/shellstring per week. In 1970 nearly all stations received a moderate (20–50 spat/shellstring) to heavy (>50 spat/shellstring) peak value and the settlement period extended over most of the summer–fall season. In 1971, no significant settlement occurred until late fall. In 1972, due to Tropical Storm Agnes, oyster settlement was at or near zero at all stations. This year marked the beginning of a major decline in spatfall in the river. The years 1973 through 1979 were characterized by a very light settlement, usually less than one spat/shellstring per week. Starting in 1980, settlement events again became more consistent (lasting throughout most of the season) and heavier (2–38 spat/shellstring per week). This increase in the number of spat in the 1980s coincided with a heavy private “planting” of a large number of small (seed) oysters on private lease grounds in the Great Wicomico; however, as these were harvested in the late 1980s and early 1990s, a further decline in the number of spat was observed. The latest signal in the river occurred during the 1997 setting season, after the artificial reef was built and stocked with brood-stock oysters. In 1997, spatfall was recorded between the end of June and the beginning of September, with a peak set occurring in mid- to late July. During this peak settlement period, spatfall ranged from 0 to 29.3 spat/shellstring per week, with the most intense sets occurring upstream or immediately adjacent to the reef (Glebe Point, Hudnall, Haynie Point, and on the reef itself; Fig. 1). Mean spatfall estimates for 1997 from shellstring data ranged from 1.4 to 43 spat/shellstring per week.

The fall dredge data taken from the VIMS database (VIMS archive) can be summarized as follows (Table 3): Between the years 1971 to 1987, the number of small oysters ranged from 90 to over 600 per bushel for all three stations (Fig. 1). During this time the number of spat per bushel ranged from a low of 0 in 1972 (the year of Hurricane Agnes) to a high of 1,900 per bushel in 1987. This year marked the beginning of a slow decrease in the number of oysters in the system. For the past 3 yrs, numbers of small oysters have ranged from 31 to 126 per bushel. 1987 also marked the beginning of essentially the absence of market-size oysters in the system. Before 1987 there were comparatively more market oysters (0–128) per bushel than after (0–22 oysters per bushel).

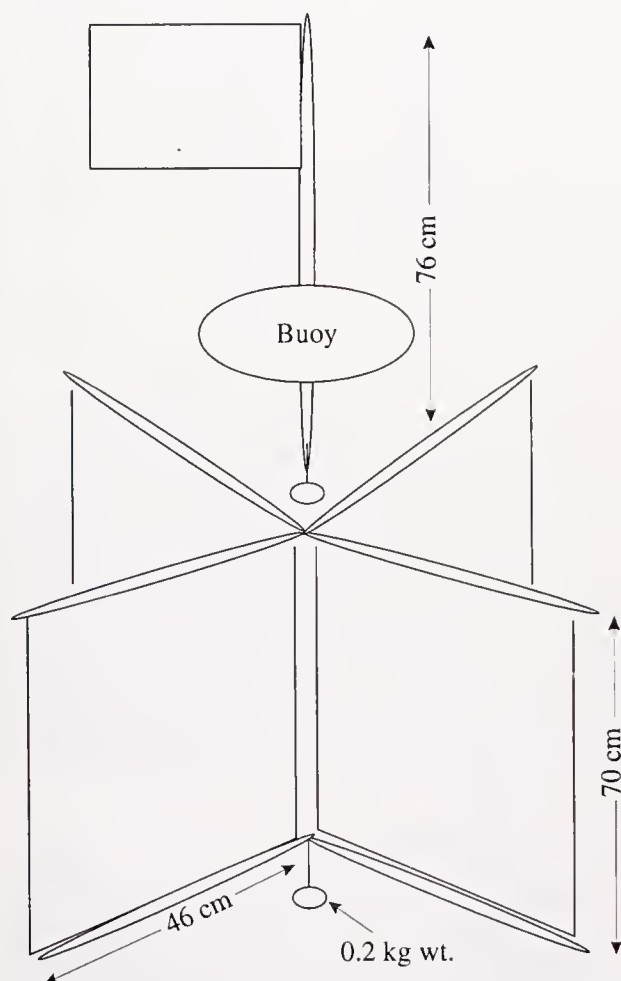


Figure 3. Design of surface drogue (drifter) used in the circulation studies.

TABLE 2.
Spatfall survey data from shellstrings reported as average spat/shellstring per week.

Year	Dameron Marsh (10)	Fleet Point (11)	Cranes Creek (7)	Haynie Point (6)	Hudnall (3)	Glebe Point (1)
1965	75.0	NS	524.0	253.0	300.0	98.0
1966	NS	NS	NS	NS	155.0	127.0
1967	8.5	NS	20.0	61.0	86.0	174.0
1968	49.0	NS	257.0	61.0	227.0	182.0
1969	8.7	24.0	45.0	112.0	89.0	36.0
1970	36.0	21.0	100.0	306.0	302.0	494.0
1971	2.6	0.9	2.4	4.4	9.6	24.0
1972	0.0	0.0	0.0	0.0	0.2	1.8
1973	2.2	1.7	0.5	1.9	0.1	0.7
1974	0.1	4.1	0.8	1.0	1.9	7.3
1975	0.1	1.0	0.4	1.1	0.1	0.2
1976	0.1	0.0	0.1	0.1	0.6	0.8
1977	0.6	1.4	1.6	0.8	2.7	0.8
1978	0.3	1.7	0.1	0.7	0.9	1.4
1979	0.6	0.9	0.1	1.3	8.3	4.1
1980	9.1	7.8	9.9	8.5	38.0	7.0
1981	2.3	1.5	3.8	17.3	21.0	83.0
1982	23.0	36.0	46.0	54.0	89.0	228.0
1983	6.8	23.0	3.8	6.9	8.6	0.4
1984	0.5	1.0	0.7	0.4	1.9	1.3
1985	5.4	49.0	4.5	4.7	8.9	6.8
1986	25.0	24.0	71.0	113.0	139.0	227.0
1987	17.0	110.0	17.0	7.6	26.0	13.0
1988	22.0	5.1	8.6	24.0	32.0	16.0
1989	3.2	4.9	5.2	10.0	16.0	4.8
1990	18.0	10.0	23.0	43.0	59.0	12.0
1991	8.5	5.2	6.6	10.0	4.0	2.3
1992	0.5	4.2	0.2	0.8	0.8	0.8
1993	0.6	1.7	0.1	1.2	0.7	0.2
1994	0.0	0.0	0.0	0.0	0.0	0.0
1995	0.0	1.3	0.2	0.3	0.1	0.9
1996	2.9	2.9	2.3	4.1	0.2	0.7
1997	1.9	4.9	1.4	6.1	43.0	24.0

Numbers in parenthesis correspond with the station IDs in Figure 1. NS means no samples were taken at that site during the corresponding year.

The number of spat recorded per bushel also started to decrease in the late 1980s. For the 5 years prior to the building of the reef, mean spatfall values were about 55 spat per bushel, whereas a mean of 155 spat per bushel was recorded for the 1997 fall survey.

Patent tong surveys revealed that in 1995 the number of market oysters ranged from 0.3 m^{-2} at Fleet Point to 1.6 m^{-2} at Sandy Point (see Fig. 1 for location of patent tong sites and Table 4 for summary of survey data). The number of small oysters ranged from 4 to 22 oysters m^{-2} with the lowest densities at Shell Bar and Fleet Point and the highest at Sandy Point. The number of spat m^{-2} ranged from 6.5 at Cranes Creek to 13.4 at Fleet Point. The overall density for the five reefs combined was 0.7 market oysters m^{-2} , 10.2, small oysters m^{-2} , and 9.7 spat m^{-2} . Whereas the mean number of market (1–3 m^{-2}) and small (9–37 m^{-2}) oysters recorded in 1997 were similar to those recorded in 1995, the number of spat were considerably higher in 1997. Spatfall estimates ranged from 4.6 m^{-2} at Ingram and Fleet Point to 103 m^{-2} at Shell Bar. The spatial pattern reflected that observed in shellstring studies with intense settlement upstream of the sand spit, and near or adjacent to the artificial oyster reef, and a trend of decreasing settlement in a downstream direction. Overall values represent a threefold increase in density of spat from 1995 to 1997.

Collectively, these survey data can be summarized as follows: Oysters were present in relative abundance in the Great Wicomico River until about 1971. The combined effects of Hurricane Agnes in 1971 and disease decimated the natural broodstock population in the system. This in turn led to a decrease in larval production and spat recruitment. For a brief time during the 1980s, oysters appeared to be returning to the Great Wicomico, but this was probably related to a large private planting of seed oysters that grew and served as broodstock for the system. Once these oysters were harvested, recruitment once again plummeted. With the building of Shell Bar reef and the addition of broodstock on the reef, recruitment once again showed an increase from previous years.

Fertilized Egg Production Estimates

Historical production of fertilized eggs in the Great Wicomico River, after correcting for disease, salinity, and fertilization efficiency, is estimated at 7.1×10^{12} . Comparable estimates for fertilized egg production on Shell Bar reef were 5.4×10^{12} embryos using size frequency distribution data from Figure 4, a salinity value of 10.8 obtained from field observations, and a fertilization

TABLE 3.

Dredge survey data reported as number of market (>76 mm), small (<76 mm), and spat per bushel.

Year	Fleet Point (7)			Whaley East (9)			Haynie Point (6)		
	Market	Small	Spat	Market	Small	Spat	Market	Small	Spat
1971	NS	NS	NS	NS	NS	NS	0.0	648.0	68.0
1972	NS	NS	NS	NS	NS	NS	NS	NS	NS
1973	128.0	260.0	0.0	NS	NS	NS	64.0	246.0	2.0
1977	60.0	88.0	82.0	48.0	182.0	24.0	38.0	112.0	156.0
1978	88.0	152.0	10.0	58.0	138.0	46.0	58.0	214.0	44.0
1979	32.0	138.0	430.0	NS	NS	NS	32.0	88.0	220.0
1980	80.0	344.0	448.0	72.0	368.0	72.0	64.0	180.0	98.0
1981	82.0	502.0	286.0	116.0	544.0	306.0	44.0	356.0	442.0
1982	30.0	414.0	1198.0	36.0	394.0	432.0	34.0	292.0	818.0
1983	28.0	476.0	124.0	32.0	188.0	74.0	10.0	208.0	78.0
1984	32.0	544.0	22.0	24.0	546.0	24.0	40.0	178.0	30.0
1985	76.0	366.0	1436.0	126.0	350.0	566.0	36.0	584.0	536.0
1986	9.5	154.0	1114.0	14.0	212.0	504.0	15.0	504.0	638.0
1987	0.0	107.0	1911.0	4.7	281.0	337.0	0.7	271.0	501.0
1988	0.0	145.0	134.0	0.0	3.0	179.0	1.3	228.0	467.0
1989	4.0	207.0	300.0	0.0	174.0	151.0	1.3	225.0	182.0
1990	11.0	297.0	473.0	0.7	275.0	44.0	0.7	141.0	397.0
1991	9.3	317.0	217.0	0.7	229.0	147.0	6.0	176.0	328.0
1992	0.0	33.0	51.0	0.0	18.0	45.0	0.0	21.0	228.0
1993	2.7	67.0	62.0	2.0	189.0	47.0	0.0	91.0	147.0
1994	5.3	150.0	7.0	2.5	114.0	6.0	0.7	153.0	7.0
1995	5.3	31.0	51.0	2.5	48.0	3.0	1.0	31.0	113.0
1996	6.0	123.0	13.0	8.0	73.0	21.0	4.5	126.0	19.0
1997	18.0	96.0	47.0	22.0	71.0	107.0	10.0	115.0	312.0

Numbers in parenthesis correspond with station IDs in Figure one. NS means no samples were taken at that site during the corresponding year.

efficiency of 29.8% based on Levitan et al.'s (1991) estimator. These calculations strongly suggest that by aggregating the brood-stock oysters into very dense populations, fertilization efficiency is greatly improved, and production of larvae on the reef is similar to that of the entire Great Wicomico system in predisease conditions.

1997 Field Studies

Surface temperature at the reef (station N1; Fig. 2) reached a maximum of 29.5°C on July 28th (Fig. 5). The difference between the surface and bottom temperature increased in a down river direction (station N1 to N3) away from the reef (Fig. 6). The maximum temperature difference occurred on July 28th for all three stations. As with the temperature, the difference in salinity between the surface and bottom water increased downstream (from N1 to N3; Fig. 6). Salinity at the three stations ranged from 12 to 18 ppt. The maximum difference encountered between surface and bottom samples was 3 ppt at station N2 and N3.

Oyster Reproductive Biology and Disease Status

At the beginning of the study, both males and females were either in the late active or ripe stage of development (Fig. 7). Evidence of spawning (spent specimens) were first seen in the July 14th samples (Fig. 7). Most of the specimen sampled were completely spawned out by early September, with a large majority of them returning to the inactive stage by the end of September.

MSX was absent in all of the oysters examined. *Perkinsus* prevalence increased from 32% in June to 100% in July and continued at that level for the remainder of the study (Fig. 8). Intensity of *Perkinsus* infection increased from June to September, with the highest percentage of highly infected oysters occurring toward the end of the study.

Plankton Studies

The number of oyster larvae observed in plankton samples ranged from a high of $37,362 \pm 4,380 \text{ m}^{-3}$ on June 23rd at station

TABLE 4.

Patent tong survey data for 1995 and 1997 reported at number of market (>76 mm), small (<76 mm), and spat per square meter.

Station	1995			1997		
	Market	Small	Spat	Market	Small	Spat
Ingram (10)	0.4	7.0	8.3	1.2	8.8	4.6
Fleet Point (11)	0.3	4.4	13.0	3.0	9.8	5.8
Cranes Creek (7)	0.8	12.0	6.5	1.9	14.0	15.0
Sandy Point (5)	1.6	22.0	10.0	0.9	37.0	62.0
Shell Bar (4)	0.1	4.1	11.0	1.6	27.0	103.0

Numbers in parenthesis correspond to the station IDs in Figure one.

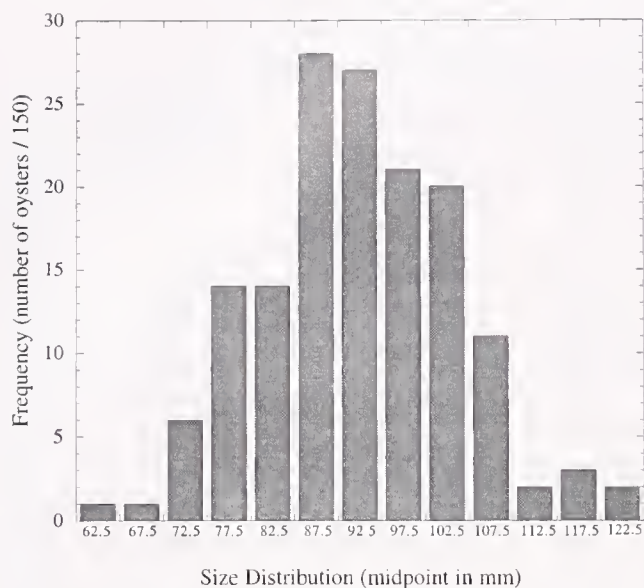


Figure 4. Size frequency distribution of broodstock oysters on Shell Bar reef ($n = 150$).

GW 2 to a low of 0 at all stations on several different sampling days. Larvae were most abundant at all stations on the 23rd and 30th of June, and on the 14th of July (Fig. 9). From the 14th of July onward, there was a continuous decrease in the number of larvae seen in the water column. Coefficient of variation for most samples was within the accepted limits of between 5 and 20% (Van Guelpin et al. 1982). Higher CVs were observed when larval abundance was below 10 m^{-3} .

The total number of larvae m^{-3} was transformed to meet the assumptions of normality and homogeneity of variance. Differences in larval abundance between tidal stage and station were

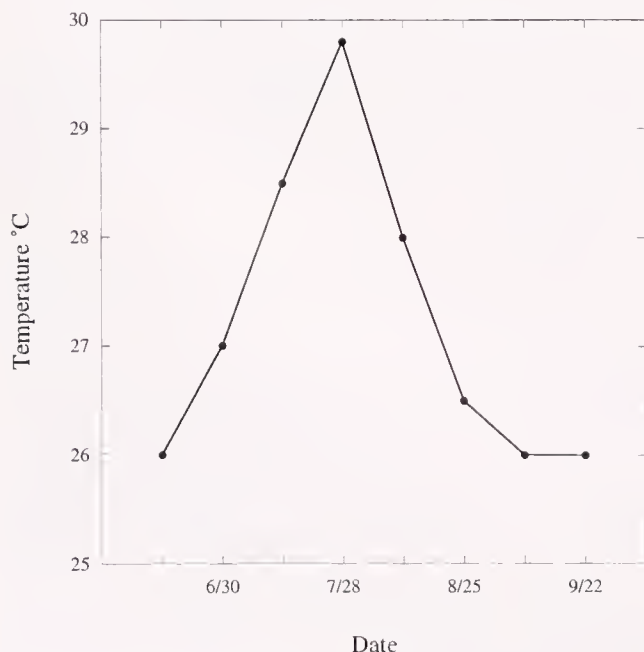


Figure 5. Surface temperature measured at station N1 from June 23 to September 22, 1997.

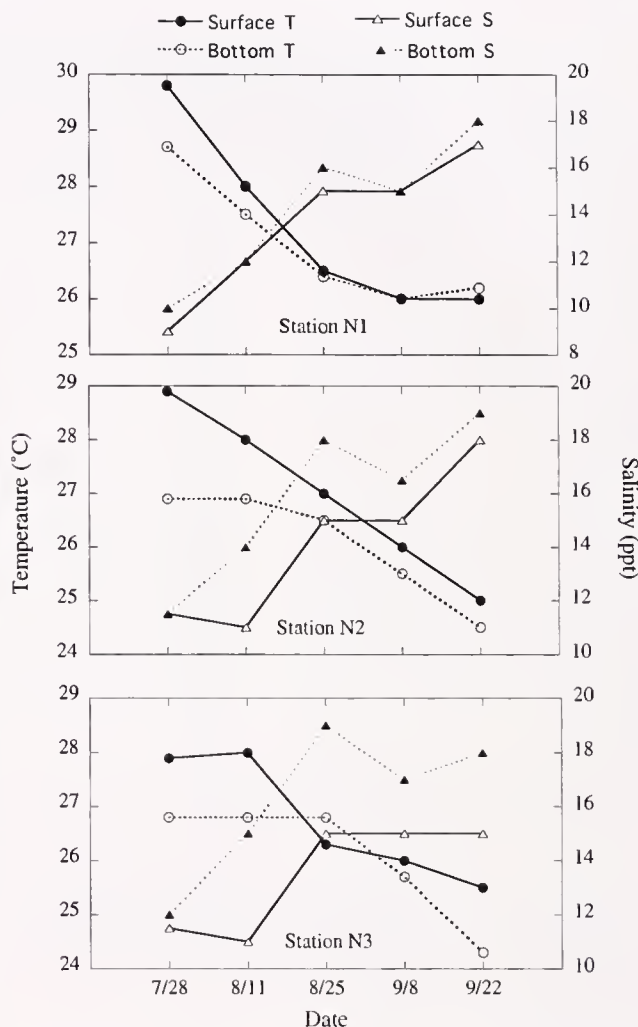


Figure 6. Surface and bottom temperature and salinity for all three stations (N1-N3) measured from July 28 to September 22, 1997.

then compared with analysis of covariance (ANCOVA) using day of the year as the covariate. The power transformation ($X' = X^{0.20}$), recommended by Downing et al. (1987) for use in estimating zooplankton populations, was used. The use of this transformation met the assumptions of homogeneity of variance, but did not meet the assumptions of normality. Given that ANCOVAs are generally robust to non-normality (Underwood 1997), this transformation was considered valid and the resulting data were utilized in performing the ANCOVA.

There was a significant difference in larval concentration between tidal stages ($p < .01$) and stations ($p < .05$), with no interaction between the two factors ($p = .55$). Student Newman Keuls (SNK) multiple comparison test for station effect showed that there were significantly more larvae at GW-1 than at the other two stations (Table 5A). There was no difference in larval abundance between GW 2 and GW 3. The SNK for tidal stage showed there were significantly more larvae during the flood tidal stage than during the ebb or slack onto flood stages. (Table 5B). No differences were found between any of the other tidal stages.

Circulation Studies

The direction, mean velocity, and distance traveled by the drifters on each sampling day, for each fix during the day, was re-

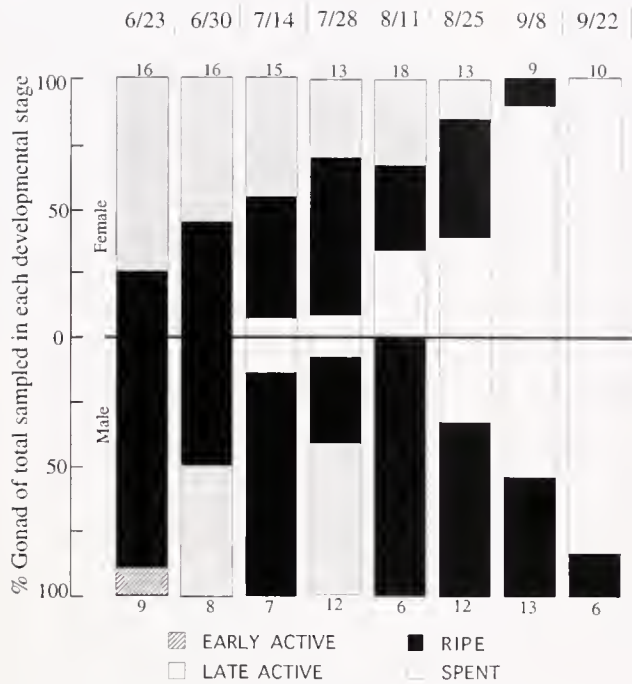


Figure 7. Seasonal changes in gonadal development by sex in *Crassostrea virginica* oysters collected on the reef from June 23 to September 22, 1997. Number of male and female oysters sampled on each day are represented by the numbers above and below each bar.

cord and/or estimated. Tidal cycle was recorded as the stage or stages of the tide occurring between a particular observation and the previous observation. For example, if the tide was ebbing for the first half of a drifter deployment, then changing to slack water for the second half, it was recorded as E-S. If the tide was flooding for the entire deployment, then tidal stage was recorded as F. Mean

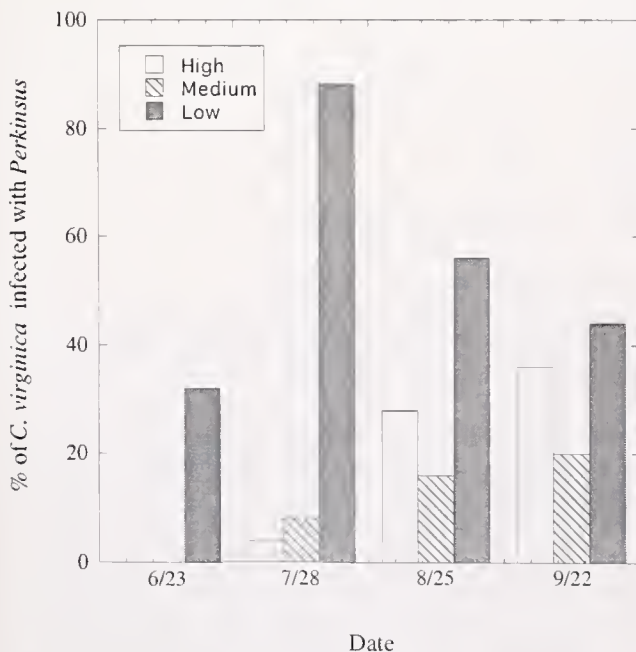


Figure 8. Progression of *Perkinsus* infections in broodstock oysters over the 1997 reproductive season (n = 25).

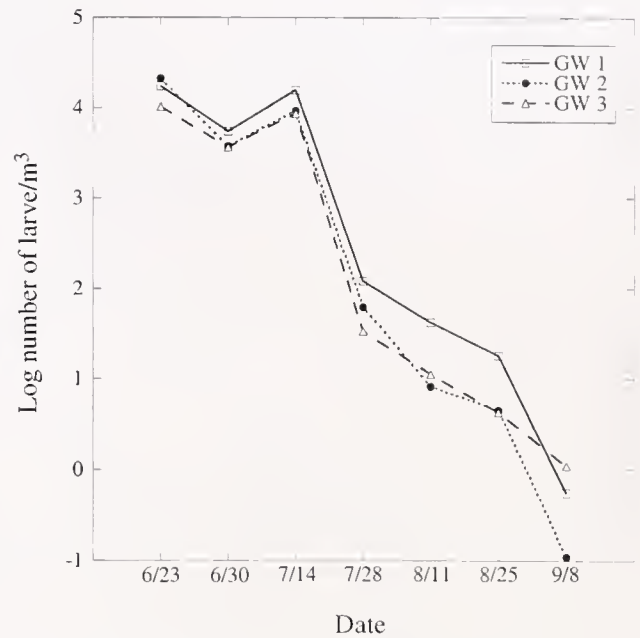


Figure 9. Log number of larvae m^{-3} averaged over each sampling day (averaged over all four stages of the tide).

velocities recorded by the drifters ranged between 0 and 15.9 cm/sec. Maximum predicted tidal current was between 10 and 20 cm/sec on all sampling days.

From the 14 drifter tracks analyzed, four patterns of movement were observed (Fig. 10A–D). Four of the 14 drifters followed the predicted tidal current, traveling downstream during the ebb tide, remaining approximately in the same place during slack water, then traveling upstream with the flooding tide. One drifter out of those four remained in the channel and drifted toward marker 9 before turning with the tide (Fig. 10A). The other 3 drifters remained in the channel, further upstream from Sandy Point (Fig. 10B provides an example).

Ten of the 14 drifters analyzed did not follow the predicted tide. Of these 10, 7 traveled downstream with the predicted tide, but turned westward, away from the channel, several hours before the predicted tide turned (see Fig. 10C for example). The other three drifters traveled downstream until they arrived in the shallow region west of Sandy Point. Despite being repositioned, they essentially remained in the same area even during a flood tide (e.g., see Fig. 10D). These patterns of movement illustrated in Figures 10C and 10D indicate that some local retention of larvae is occurring upstream of Sandy Point.

TABLE 5.

SNK multiple comparisons test on larval abundance ($p = .05$).

A. Ranking of Larval Abundance by Station				
Station	High GW-1	Low GW-2	Low GW-3	
B. Ranking of Larval Abundance by Tidal Stage				
Tidal stage	High Flood	Slack-ebb	Ebb	Low Slack-Flood

Stations/tidal stages underlined by the same line are statistically the same.

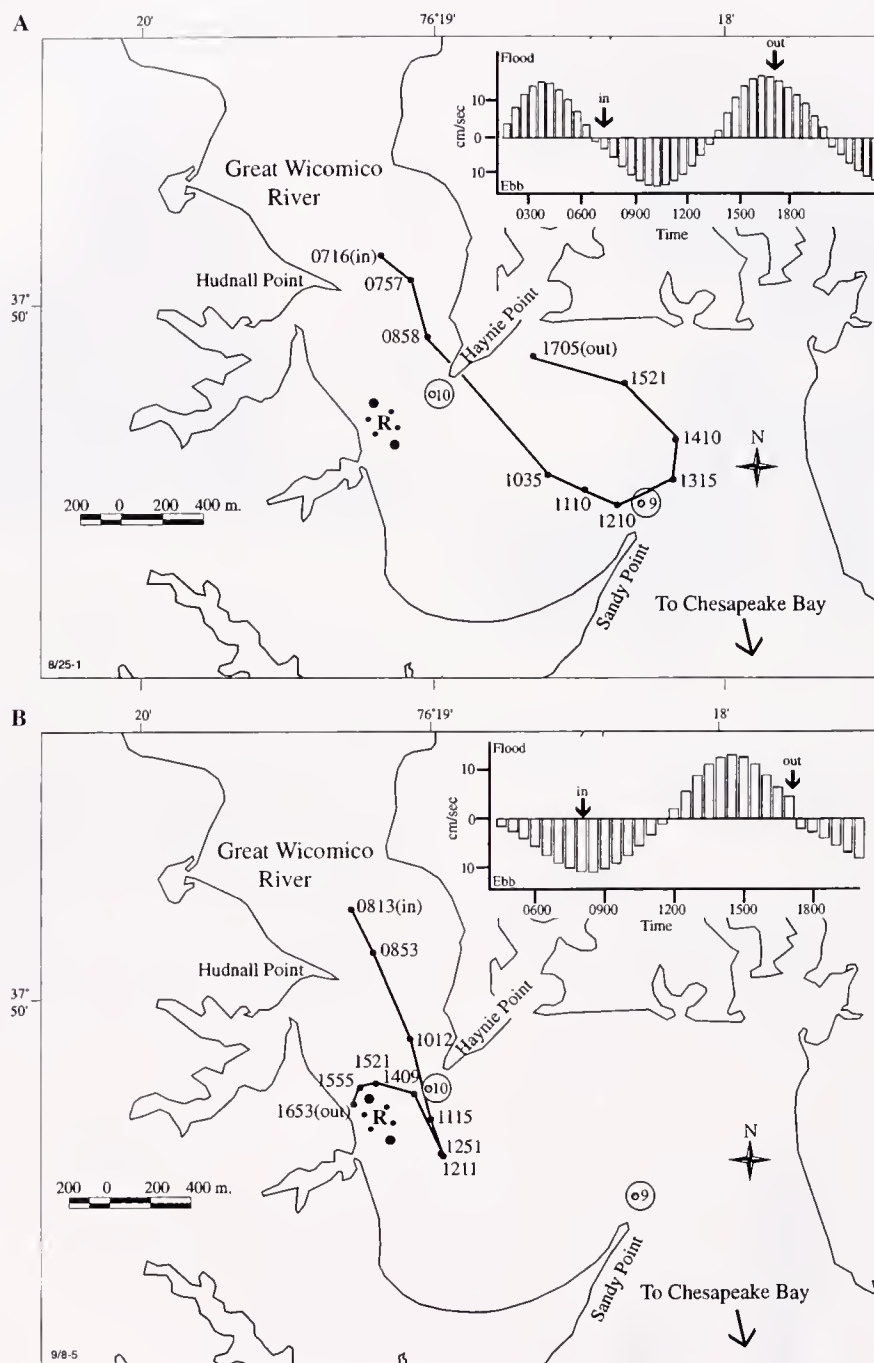


Figure 10. Representative examples of the four patterns of drifter tracks (A–D) observed over the course of the study. Inset shows predicted tidal currents for Sandy Point, with arrows representing approximate times of deployment (in) and retrieval (out) of the drifters. Time is reported in Eastern Standard (E.S.) Military time. R denotes the location of the reef and 9 and 10 mark the main channel in the river.

DISCUSSION

Egg Production

Egg production estimates for broodstock oysters planted on the reef were found to be similar to estimates of production seen throughout the entire system in historic times (when oysters were abundant in the river). Though the estimates are similar they should be viewed with caution because of our inability to offer better values for disease- and salinity-related modifiers of fecundity. These are both discussed in Mann and Evans (1998) and are

widely acknowledged in the literature as having major effects on the bioenergetics of oysters, and yet they are still poorly described in a quantitative sense.

The model used in the calculation of fertilization efficiency taken from Levitan's (1991) work on echinoderms, involves a series of assumptions concerning synchrony and completeness of spawning, half-life of gametes in the water column, dispersal or dilution, and probability of fertilization given absolute concentrations of sperm and eggs. There is a notable absence of models in the literature describing fertilization efficiency for sessile bivalves. The employment of the Levitan model in the current application

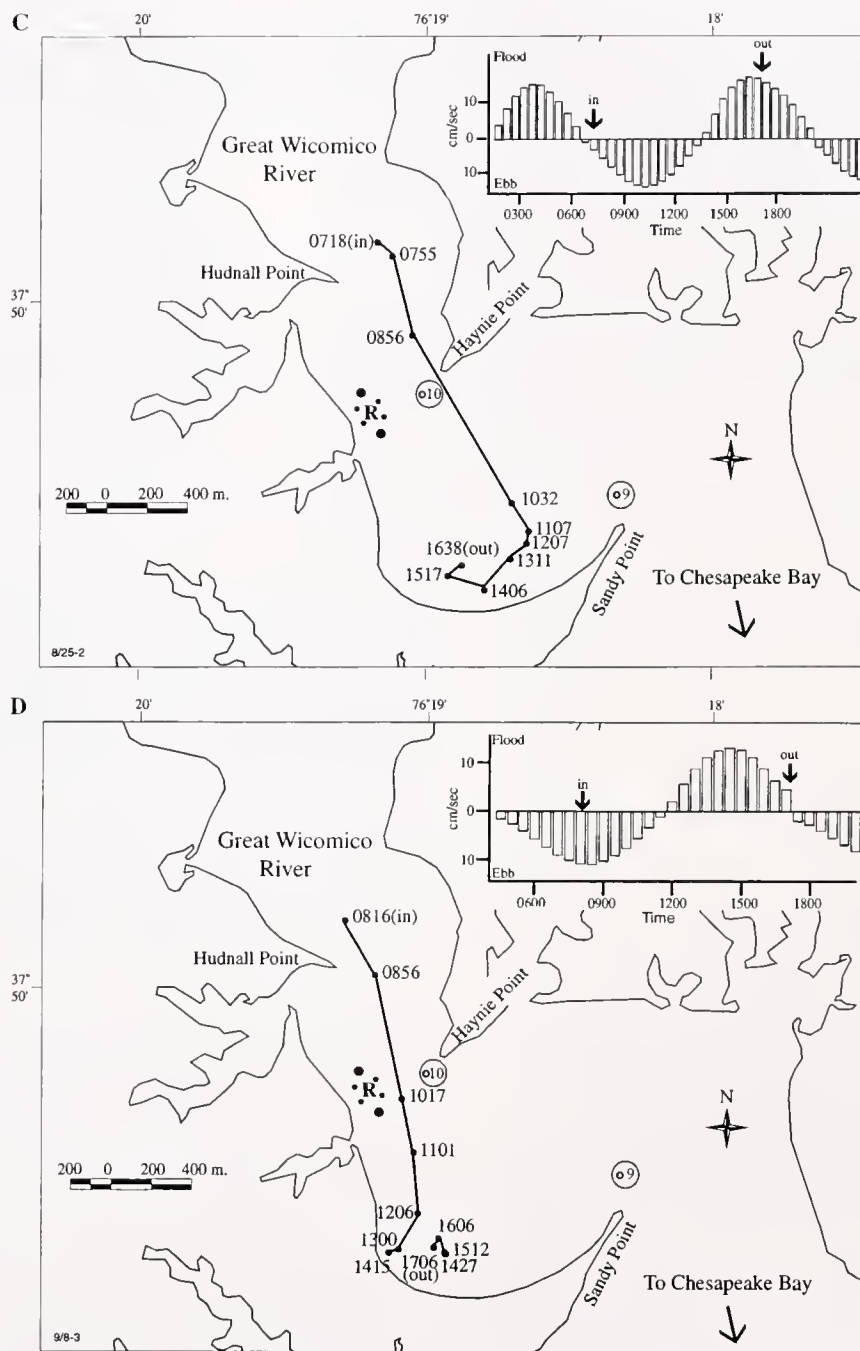


Figure 10. Continued.

was deemed reasonable based on the similarities in small scale hydrodynamic conditions seen in both Levitan's model and estuarine oyster reefs. Other options for models are discussed by Levitan et al. (1991), Oliver and Babcock (1992), and Benzie et al. (1994). Based on the hydrodynamics of the Great Wicomico, contrasting models such as the one for high-energy environments seen in Denny and Shibata (1989) are inappropriate for use in the Great Wicomico system. The current model assumes synchrony in spawning throughout the entire oyster population; however, local synchrony is more appropriate when discussing these populations. The lack of synchrony throughout the population is demonstrated by the variation in developmental stage seen in this and other studies (e.g., Haven and Fritz 1985) that observe spatial variation

in settlement. Localized synchrony in spawning on the other hand is highly probable, so the cumulative effect of these localized events approximates in magnitude that of a single synchronous spawning in the entire population. In other words, the cumulative output from multiple spawnings that occurs throughout the reproductive season (typically a maximum of 2–3 per season based on historical spatfall observations) are within an order of magnitude of the single synchronous spawning event estimate of production.

Oyster Reproductive Biology and Disease Status

Both histological evidence and the presence of significant number of larvae in plankton samples indicate spawning of the trans-

planted broodstock oysters on Shell Bar reef. Based on the larval abundance data, and an estimated 14 to 21 days spent in the water column by *C. virginica* larvae, inferences can be made about the timing of spawning. Oysters from the reef probably spawned continuously from mid-June to the end of July, with a few late spawnings occurring in the beginning of August. *C. virginica* populations in the Chesapeake Bay have been reported to spawn from June to August (Kennedy and Krantz 1982). Gonad data from broodstock oysters on the reef support this observation. Though histological evidence of spawning was not observed until mid-July, ripe males and females were observed by mid-June. Small sample numbers (25 oysters per sampling day compared with a resident population of approximately 1.1×10^6 individuals) and spatial asynchrony of spawning on the reef may have confounded the definition of the first spawning event.

Perkinsus infections progressed through the broodstock population throughout the summer, but did not result in catastrophic mortalities. The effect of *Perkinsus* on adult oysters, mainly reduced fecundity, increases as intensity of the disease increases (Choi et al. 1994). In the broodstock oysters, intensity was highest toward the end of the sampling season, after the majority of the spawning had already taken place, suggesting that disease was probably not a limiting factor in the production of larvae.

Plankton Studies

Larval concentrations at the surface were found to be significantly higher during the flood tidal stage, suggesting that the larvae are acting as more than just passive particles. Larvae are capable of depth regulating with changes such as density and salinity, associated with a change in tidal cycle, such that a net transport of larvae upriver is possible (Hidu and Haskin 1978, Mann 1988). On most of the sampling days in this study, there was some stratification occurring in the water column at stations N2 and N3 (in the channel). The lack of physical stratification at N1 may have been due to the shallower depths at this station. Despite the lack of stratification at N1, the suggestion of larval depth regulation with the changes in tidal stage are still supported by the absence of a significant interaction between tidal stage and station.

It has been proposed that larval retention in an estuarine system and subsequent movement upstream is brought about through a combination of passive transport and active larval swimming (Carriker 1951, Kunkle 1957, Haskin 1964). Oyster larval concentrations reported in the literature over the past 75 years range from 12 m^{-3} to 660,000 m^{-3} (Table 6). Wood and Hargis (1971) report larval abundance at the surface during maximum flood tide in the James River, with concentrations of larvae ranging between 300 and 800 m^{-3} . They found that minimum concentrations ($<100 m^{-3}$) were encountered during slack water, following the ebb tide. The highest larval concentrations reported in the literature were recorded in Delaware Bay as 660,000 m^{-3} (Nelson and Perkins 1931) and 125,500 m^{-3} (Nelson 1927). These numbers are extremely high when compared with concentrations found in this study, but the date of the observations must be taken into account. In a more recent study by Mann (1988) in the James River, much lower concentrations of between 12 and 113 m^{-3} were reported. The concentration of larvae found, reported in this study, is extremely high when compared with other reports made for years after the onset of disease and decimation of broodstock oyster populations in the Chesapeake Bay subestuaries. Though not of the same order of magnitude seen in historical times, the present report of concentration of larvae in the Great Wicomico is still several

TABLE 6.
Oyster larval concentrations reported in the literature over the past century.

Larval Concentration m^{-3}	Location	Year	Source
125,000	Barnegat Bay, NJ	1927	Nelson, 1927
660,000	Barnegat Bay, NJ	1931	Nelson and Perkins, 1931
16,680	Lanoka Lagoon, NJ	1938	Carriker, 1951
13,360–37,300	Great Bay, NJ	1939	Carriker, 1951
625–2400	Delaware Bay, NJ	1656	Haskin, 1964
300–800	James River, VA	1965	Wood and Hargis, 1971
12–113	James River, VA	1985	Mann, 1988
17,000–37,500	Great Wicomico River, VA	1997	This study

orders of magnitude higher than that found in the James River, which is considered to be the most important oyster-producing river in the Chesapeake Bay. A few James River reefs have similar densities of broodstock oysters as that found on Shell Bar Reef (see Table 1 of Mann and Evans 1998), but the difference in larval abundance probably lies in the differences in size and fecundity between the two broodstocks. The typical size of oysters found in the James is between 45 and 60 mm, with only a few reaching above 85 mm. In contrast, typical oysters found on Shell Bar Reef are between 85 and 95 mm. Given that fecundity and size have a nonlinear relationship (Mann and Evans 1998), small differences in mean broodstock size, and hence fecundity, can lead to vast differences in larval production.

Circulation Studies

Estuarine circulation can be a critical component of larval retention (Pritchard 1953, Ruzecki and Hargis 1989). The general trend of the current tracks in this study suggest that circulation in the system is favorable for the retention of larvae. The majority of the tracks had a tendency to turn away from the channel, prior to tidal current change, thus the drifters remained upstream of Sandy Point, in the general area of the reef. In several instances, initial drifter movement was downstream in the channel. On only one occasion, however, did the drifter continue traveling in the channel toward the mouth of the river. Settlement data were in concordance with circulation observations in that higher settlement was found upriver of the sand spit in both the patent tong and shellstring surveys, suggesting that some local retention of larvae produced by the broodstock oysters on the reef was occurring. The number of spat m^{-2} on the bottom recorded from the patent tong survey were at least three times higher at stations upstream compared with the stations downstream.

The Great Wicomico River has historically been termed a "trap-type estuary," along with the Piankatank River, also in Virginia and the St. Mary's River in Maryland (Manning and Whaley 1954). Andrews (1979), defines a trap-type estuary as one that has a low-flushing rate, small tidal amplitudes, and restricted entrances. Though these characteristics are important, local circulation, dictated by both topography and tidal currents, has been shown to be an important component of larval retention (Carter 1967). Larval retention is not, however, limited to trap-type estuaries. The James River, for example, has proved to be a good

seed-producing river, with larvae that are produced in the lower reaches being moved upstream and subsequently settling on upstream oyster beds (Ruzecki and Hargis 1989, Mann 1988). The important feature of retention in the James River is the tidal-related gyre-like circulation in Hampton Roads. Thus, retention of larvae can occur in estuaries that have characteristics in direct contrast to the "typical" trap-type estuary.

Impact of Broodstock Seeding from a Management Perspective

The demonstrated positive impact of broodstock addition to the Great Wicomico reef prompts the question as to the efficacy of this approach for widespread restoration efforts in the Chesapeake Bay and elsewhere. In comparison with prior reef construction efforts, the addition of broodstock to the reef in the Great Wicomico River approximately doubled the initial monetary investment, however, the ecological impact on replenishment was rapid in comparison. In just 1 y observed settlement of spat was comparable to historic conditions. Increased spatfall in 1997 has stimulated a resurgence of interest in deployment of substrate on private leased oyster grounds in the Great Wicomico in 1998 in anticipation of continued high recruitment. A blanket endorsement for the construction and broodstock enhancement of reefs should still, however, be avoided. Location of any proposed reef construction is critical to its possible success. Though the typical definition of a trap-type estuary is useful in general location, knowledge of historic oyster reef topography can be critical at a finer spatial scale. Indeed, our

study supports the use of both historical data sets and circulation studies of a target system as a common precursor to management decisions involving reef construction. Location definition should be followed by numerical estimation of optimal stocking density. The current study stands as an example of how a modest (compared with historical numbers) number of large oysters planted at a high density of 300 m⁻² can have an immediate impact in an effort to restore decimated oyster populations to historic conditions.

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STUDIES ON TRIPLOID OYSTERS IN AUSTRALIA. X. GROWTH AND MORTALITY OF DIPLOID AND TRIPLOID SYDNEY ROCK OYSTERS *SACOSTREA COMMERCIALIS* (IREDALE AND ROUGHLEY)

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ABSTRACT Growth and mortality of triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley) were compared with those of sibling diploids at 13 oyster farms in 10 estuaries in New South Wales (NSW). Although results varied between farms, after 2–2½ years on commercial oyster leases, triploids were on average 30.7% heavier and 8.6% larger in shell height than sibling diploids. Seven of the 13 farms had triploids with a mean weight of at least market size (40 g) after 2–2½ years while no farms had diploids of the same mean weight. The growth advantage of triploids began to be expressed at specific sizes rather than ages, specifically at a mean whole weight above 5–10 g or shell height of 30–40 mm. In general, triploids appeared to grow faster compared with diploids at higher water temperatures. Although readily accepted by processors during cooler seasons, triploid Sydney rock oysters developed a patchy, discoloration of the gonad during warmer months that may affect marketability during summer. Mortality of triploids was significantly ($p < .01$) lower than that of diploids at 6 of the 13 farms and did not differ ($p > .05$) at 6 of the remaining 7 farms. The growth coefficients were lower and mortality higher for wild-caught diploids than both hatchery-reared diploids and triploids. Triploid Sydney rock oysters were found to offer significant commercial advantages over diploid oysters because of faster growth rates and lower mortality when grown under commercial oyster farming conditions across a range of estuaries in NSW.

KEY WORDS: Growth, farming, oysters, triploid, mortality

INTRODUCTION

Sydney rock oysters generally take from 3 to 4 years to reach marketable size (Nell 1993) compared with 1½–3 years for Pacific oysters, *Crassostrea gigas*, Thunberg (Graham 1991) in Australia. Increasingly, Pacific oysters (predominantly from Tasmania and South Australia) are taking over traditional Sydney rock oyster markets. Adoption of triploid technology may help New South Wales' (NSW) farmers to remain competitive against industries based on the faster growing Pacific oyster. Nell et al. (1994) compared the performance of triploid and diploid Sydney rock oysters in a small scale trial in Port Stephens, NSW over 2½ years. At the end of the study, the triploids were on average 41% heavier and reached market size 6–18 months earlier than their diploid siblings. However, results varied among the four sites tested within the one estuary (range 32–48.8% difference in whole weights of diploids and triploids). The benefits of triploidy are known to be influenced by environmental factors such as food availability (Davis 1989a) and temperature (Davis 1989b, Shpigel et al. 1992). Oyster farming in NSW is practiced throughout the coastal areas of the state, across a range of environmental conditions (particularly temperature). At the conclusion of the preliminary study, there were no commercial bivalve hatcheries operating in NSW. To commercialize production and farming of triploid Sydney rock oysters, potential hatchery operators needed to be assured that the benefits of triploidy could be extended to other oyster-growing estuaries, using commercial farming methods. In this study, to enable the commercialization of triploid technology, we compared the growth and mortality of diploid and triploid Sydney rock oysters grown on oyster farms throughout NSW by commercial oyster farmers. This

study also provides a comparison of normal, diploid oyster growth across a range of NSW estuaries.

METHODS

Induction of Triploidy and Ploidy Determination

Triploid (300×10^6) and diploid (60×10^6) sibling larvae were produced in February 1994 from 48 female and 6 male oysters from Port Stephens, NSW. Triploidy was induced in zygotes using the method described in Nell et al. (1996) with a cytochalasin B (CB) concentration of 1.25 mg/L. Larvae were stocked directly into 20,000 l larval rearing tanks (three triploid and one diploid).

Percentage triploidy was determined by direct chromosome counts at 4 h in embryos (Nell et al. 1996) and by flow cytometry for shelled larvae and spat.

Larvae and Spat Rearing

Larvae were reared using standard hatchery techniques for Sydney rock oysters (Frankish et al. 1991). The three triploid treatment tanks were combined by day 5 due to low survival and similar percent triploid readings (78.3–81.5%). Screening at water changes was conservative to allow for initial slow growth of triploid larvae following exposure to CB (Wada et al. 1989). Larvae were settled on ground scallop shell between days 18 and 22 and reared in the hatchery for 5 weeks before being transferred to outdoor upwelling units. Spat were on-grown to a size of 7–10 mm.

Oyster Management

Thirteen commercial oyster farmers in 11 different estuaries throughout NSW (Fig. 1) from Pambula Lake (36°58'S, 149°54'E) to Hastings River (31°25'S, 152°55'E) were each sold 25,000 diploid and 25,000 sibling triploid spat between July and

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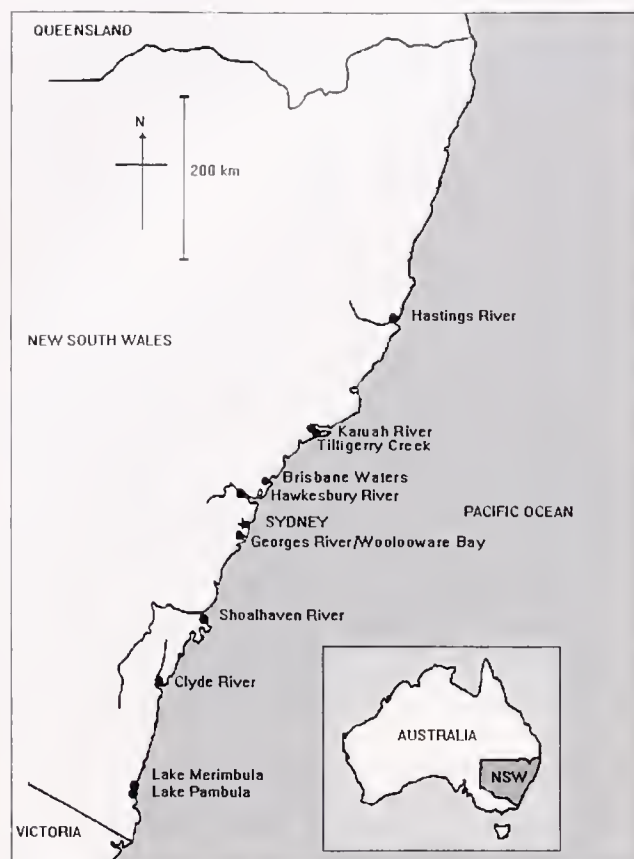


Figure 1. Estuaries in NSW where farming sites were located (adapted from Holliday et al. 1988).

November 1994. Rather than supplying oysters matched for initial size (i.e., which would risk matching fast diploids with slow triploids), oysters were supplied to farmers as they reached a grow-out size of 7–10 mm (shell height). Mean whole weight of spat supplied to farmers ranged from 0.07 ± 0.02 to 0.11 ± 0.05 g and 0.07 ± 0.02 to 0.10 ± 0.03 g (mean \pm SD; $n = 400$) for diploids and triploids, respectively. Mean shell height ranged from 8.40 ± 0.82 to 9.85 ± 1.68 mm and 8.22 ± 0.83 to 9.73 ± 1.05 mm (mean \pm SD; $n = 400$) for diploids and triploids, respectively. All farmers were encouraged to use standardized stocking densities throughout the experiment of 2 L for cylinders and 50% coverage for tray culture (Nell 1993). However, in the colder South coast areas (i.e., Lake Pambula and Lake Merimbula, where conditions produce limited oyster growth in cylinders at these densities) they were reduced. Baskets (Maguire et al. 1994a) were stocked at between 1 and 2 L. Table 1 shows a summary of culture methods used at each farm. Oysters were grown by the farmers using standard commercial oyster farming techniques, and culture equipment was provided by farmers. For this reason culture trays, baskets, etc. could not be standardized across all estuaries, however, diploids and triploids at each farm were grown under the same conditions. The normal farming practice of grading oysters into several size grades, when there is a large range of sizes to allow growth of the smaller oysters without competition from the larger grades, was minimized where possible to make comparisons between ploidy levels and farms simpler. However, as this was a study based on commercial farming methods, and large size ranges developed at least at some stage during the course of the experiment [at all sites except for Tilligerry Creek, Port Stephens (farm 1) and Woolloomare Bay], oysters were graded when necessary. Grades were recombined when there was no longer a difference in size.

Oysters from Georges River (farm 2) and Lake Pambula were

TABLE 1.

Culture methods for diploid and triploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales from July 1994 to November/December 1996.

Estuary	Month Supplied (1994)	Nursery Culture		Growout	
		Culture Method	Stocking Density	Culture Method	Management
Hastings River	July	Subtidal trays	50% coverage	Subtidal trays	Oysters dried out 1–7 days every 4 weeks
Tilligerry Creek (farm 1)	"	Intertidal trays	50% coverage	Intertidal trays	Moved during winter
Tilligerry Creek (farm 2)	August	Intertidal trays	50% coverage	Intertidal trays	Moved during winter
Karuah River	September	Cylinders	21	Baskets	
				Intertidal trays	
Georges River (farm 1)	"	Cylinders	21	Baskets	Moved during winter
				Intertidal trays	
Georges River (farm 2)	"	Cylinders	21	Baskets	Moved during winter
				Intertidal trays	Divided between 3 sites
Hawkesbury River	October	Cylinders	21	Intertidal trays	
Woolloomare Bay	"	Intertidal trays	50% coverage	Intertidal trays	Moved during winter
Clyde River	"	Intertidal trays	50% coverage	Intertidal trays	Moved during winter
Lake Merimbula	"	Cylinders	1–21	Intertidal trays	Raised during winter
Lake Pambula	"	Cylinders	0.5–21	Intertidal trays	Divided between 3 sites
		Trays	50% coverage		
Brisbane Waters	November	Cylinders	21	Baskets	Large grades moved between large cylinders and trays
				Intertidal trays	
				Large cylinders ^a	
Shoalhaven River	"	Cylinders	0.5–21	Intertidal trays	After 9 months on intertidal trays
				Subtidal trays	Transferred to subtidal culture for 6 months

^a 200 l rotating cylinders with 12 mm mesh, similar to the smaller Stanway® cylinders.

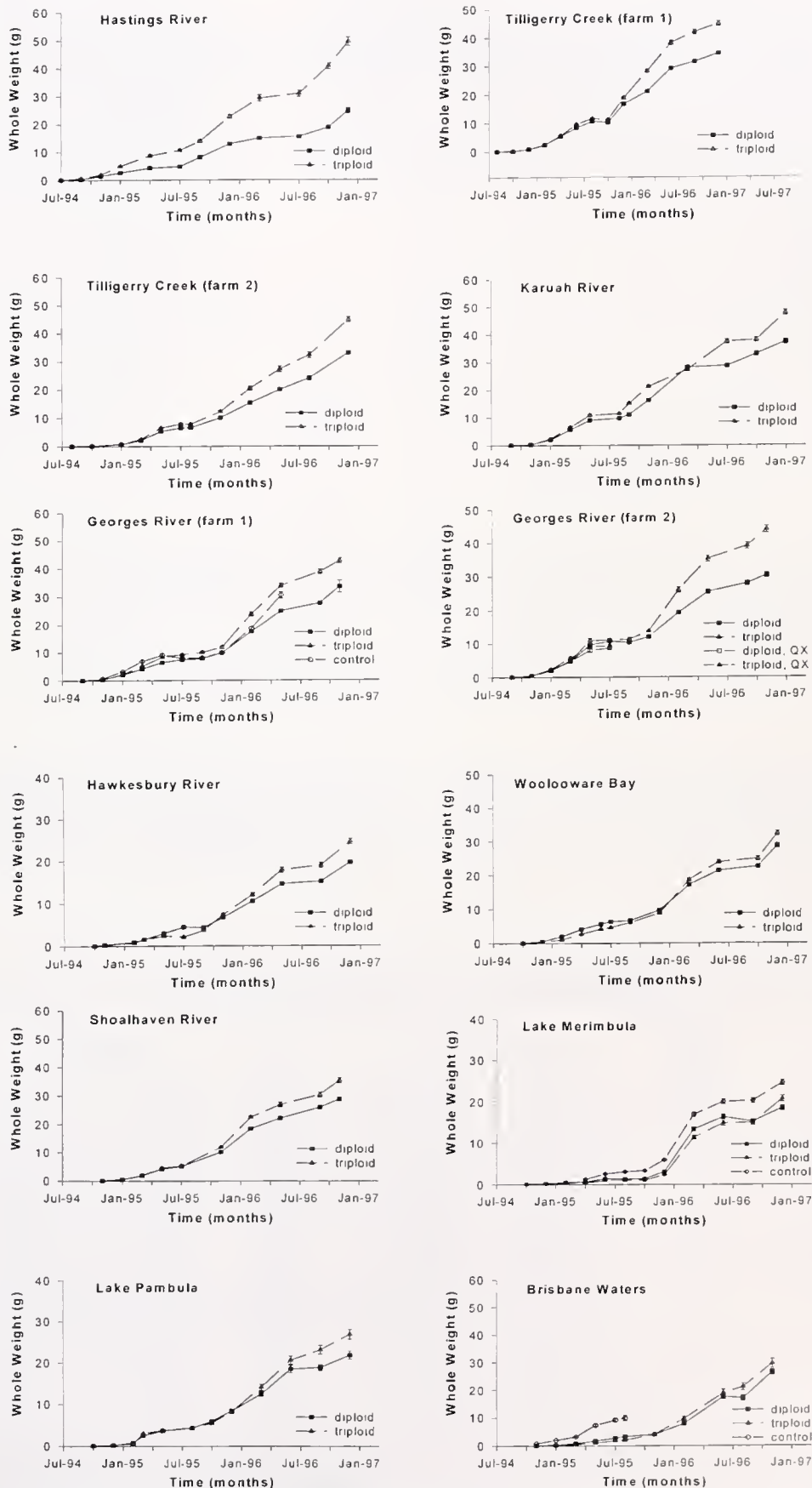


Figure 2. Whole oyster weight (g) of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, grown by commercial oyster farmers in NSW from July 1994 to December 1996. Mean \pm 95% confidence intervals.

TABLE 2.

Time^a taken (months) to reach a whole weight of 10, 20, 30, and 40 g by diploid and triploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales.

Estuary	Site	10 g		20 g		30 g		40 g	
		Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
Hastings River ^{b,c}		15	12	28	16	—	22	—	27
Tilligerry Creek (farm 1)		12	11	18	17	23	20	—	24
Tilligerry Creek (farm 2)		14	13	20	17	26	22	—	26
Karuah River		11	8	15	14	23	19	—	26
Georges River (farm 1)		14	11	18	16	25	19	—	25
Georges River (farm 2)	1	12	11	17	16	26	18	—	25
	2	8	7	17	16	26	18	—	25
Hawkesbury River		14	15	19	18	—	27	—	—
Woolooware Bay		14	15	19	18	—	25	—	—
Clyde River ^b		14	13	17	14	26	19	—	26
Lake Merimbula ^b		16	17	—	26	—	—	—	—
Lake Pambula ^b	1	17	16	25	20	—	—	—	—
	2	16	15	27	23	—	—	—	—
	3	15	15	20	19	—	—	—	—
Brisbane Waters ^b		15	16	22	20	—	24	—	—
Shoalhaven River		12	11	16	14	—	22	—	—
Average time ^d		14	13	(19)	17	(25)	(20)	—	(26)

^a Actual time taken, estimated from graph of whole weight against time: — represents oysters that have not reached set size.

^b A weighted mean of more than 1 size grade was calculated.

^c Subtidal culture.

^d Mean time for all farms; months in brackets do not include data from slower growing sites where times are not available for that set size and for both ploidy types.

divided among three sites when they reached a large enough volume (after 2 and 6 months, respectively). Oysters from Georges River (farm 2) were divided between one upriver lease and two downriver leases. During the first year, oysters from the upriver site suffered high mortality with symptoms consistent with the

disease QX. After 12 months this site was no longer included in the study because of very poor survival, and the remaining two downriver sites were combined. QX disease, which is caused by the protistan parasite *Marteilia sydneyi*, spread to the Georges River in 1994 (Adlard and Ernst 1995).

TABLE 3.

Whole weight and shell height of diploid and triploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales from July 1994 to November/December 1996.

Estuary	Month Supplied (1994)	Whole Oyster Weight (g)			Shell Height (mm)		
		Diploid	Triploid	Difference (%) ^a	Diploid	Triploid	Difference (%) ^b
Hastings River ^{b,c}	July	24.9 ± 0.5	49.4 ± 0.8	98.9	60.4 ± 0.5	69.6 ± 0.5	15.2
Tilligerry Creek (farm 1)	"	34.4 ± 0.3	44.8 ± 0.5	30.1	68.0 ± 0.3	74.0 ± 0.4	8.9
Tilligerry Creek (farm 2)	August	33.0 ± 0.3	44.8 ± 0.5	35.8	69.6 ± 0.3	75.1 ± 0.4	7.9
Karuah River	September	37.4 ± 0.4	47.8 ± 0.5	28.0	66.1 ± 0.3	69.8 ± 0.3	5.5
Georges River (farm 1)	"	33.5 ± 1.1	42.6 ± 0.5	27.1	60.7 ± 0.8	66.6 ± 0.3	9.7
Georges River (farm 2)	"	30.3 ± 0.3	43.9 ± 0.5	44.9	60.5 ± 0.3	68.1 ± 0.4	12.5
Hawkesbury River	October	19.6 ± 0.2	24.6 ± 0.3	25.3	52.2 ± 0.3	59.0 ± 0.4	13.1
Woolooware Bay	"	28.7 ± 0.3	32.3 ± 0.4	12.6	58.8 ± 0.3	62.4 ± 0.3	6.0
Clyde River ^b	"	30.3 ± 0.4	39.9 ± 0.6	32.0	63.3 ± 0.4	68.7 ± 0.5	8.6
Lake Merimbula ^b	"	18.7 ± 0.3	20.9 ± 0.4	11.8	51.7 ± 0.4	56.6 ± 0.4	9.5
Lake Pambula ^{b,d}	"	21.9 ± 0.5	26.9 ± 0.6	23.0	57.0 ± 0.6	62.2 ± 0.6	8.4
Brisbane Water ^b	November	26.7 ± 0.5	28.2 ± 0.7	5.6	63.3 ± 0.5	63.7 ± 0.7	0.8
Shoalhaven River	"	28.8 ± 0.3	35.3 ± 0.5	22.6	63.3 ± 0.3	66.9 ± 0.4	5.6
Average		28.3	37.0	30.7	61.1	66.4	8.6

^a Difference (%) = (triploid - diploid)/diploid × 100.

^b A weighted mean ± SE of more than 1 size grade was calculated for mean whole weight and shell height.

^c Subtidal culture.

^d Oysters divided among three sites.

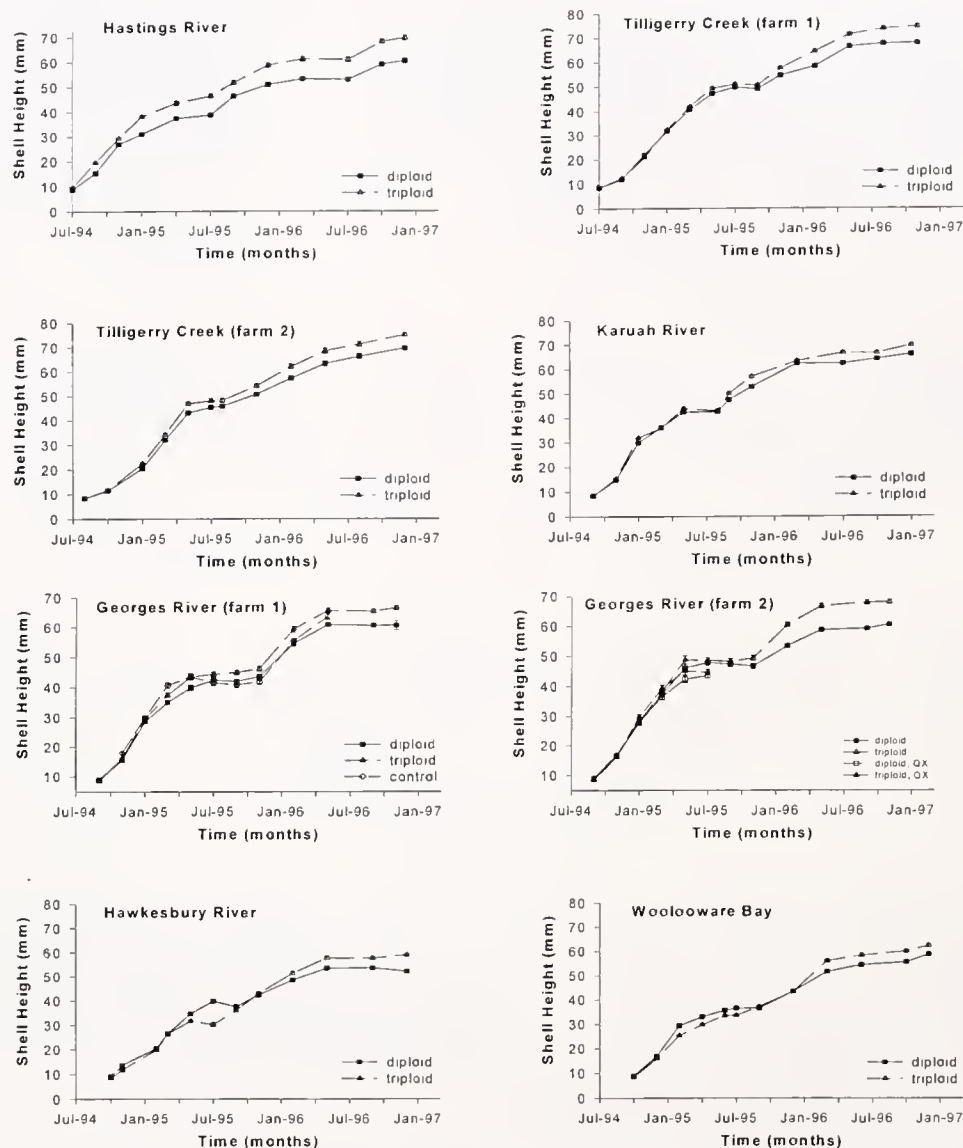


Figure 3. Shell height (mm) of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, grown by commercial oyster farmers in NSW from July, 1994 to December 1996. Mean \pm 95% confidence intervals.

As is commercial practice, oysters located in estuaries subject to the disease winter mortality were moved where possible (Tilligerry Creek: farms 1 and 2; Georges River: farms 1 and 2; Woollooware Bay and Clyde River) to a lease further upstream or to a higher rack (Lake Merimbula) during winter. Winter mortality is caused by the protistan parasite *Mikrocytos roughleyi* (Farley et al. 1988).

A further comparison of a cylinder of "wild-caught" single-seed (Nell 1993) diploid oysters with the hatchery-produced diploids and triploids was set up at Georges River (farm 1), Lake Merimbula and Brisbane Waters.

Sampling Strategy

A subsample of 400 of each type of oyster was measured for whole weight and shell height before being supplied to farmers, then every 2 months thereafter at each farm until November 1995 when the sampling frequency was changed to every 3 months.

Where several grades were present, a smaller sample was measured for each grade, i.e., 400–600 total per ploidy type and a weighted mean was determined. Samples for individual height and weight measurements were taken from a random 10% of stock brought in every 2–3 months. For this reason, mortalities were not removed during the course of the experiment (i.e., to maintain similar densities of oyster shells and percentage dead between sampled trays and trays remaining on leases). Mortality was assessed from a subsample and returned to trays for a final count at the end of the experiment.

Total weight of stock was measured every 2 months for the first 6–9 months to assess losses and the proportion in each grade. As handling became more difficult with the large increase in volume during the first year (after March 1995), farmers were given the choice of bringing in either 25% of stock or everything every 6 months to find the total weight of all oysters. Weights of dead oysters and overcatch were accounted for when total weights were measured.

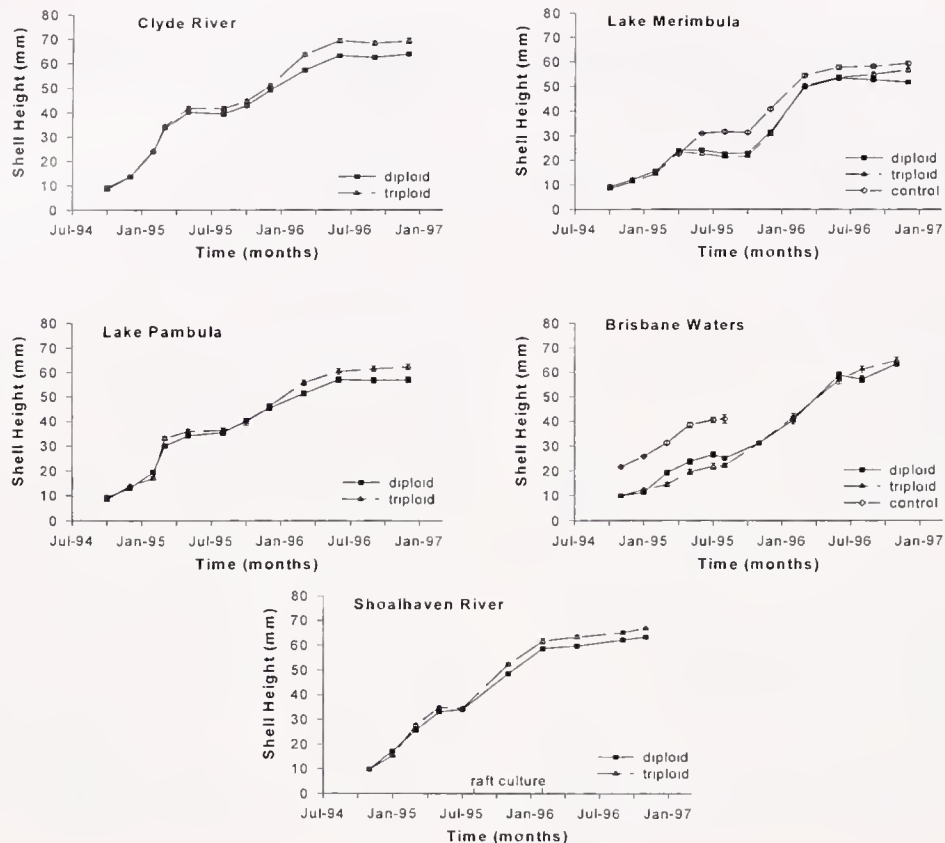


Figure 3. Continued.

Salinity and temperature data were measured weekly by farmers using a thermometer and hydrometer.

Statistical Analyses

All whole oyster weight and shell height data are presented in figures as means \pm 95% confidence intervals. Weighted means of whole weight, shell height, and mortality data were calculated for farms with more than one size grade or site:

$$\bar{X}_w = \frac{\sum_{i=1}^n W_i \bar{X}_i}{\sum_{i=1}^n W_i}$$

where \bar{X}_w is the weighted mean calculated from the mean, \bar{X}_i , of each n grades, and each \bar{X}_i weighted by a factor W_i (Sokal and Rohlf 1995). The total number in each grade was used as a weighting factor and was estimated from the total weight of all oysters for each grade divided by the mean weight of individual oysters for that grade.

Growth coefficients (G_{90}) were calculated from comparisons of hatchery stock with wild-caught diploids to account for differences in initial whole weights and in the duration of the experiment (Spencer and Gough 1978):

$$G_{90} = \frac{90}{\text{Duration (days)}} \times \ln \left(\frac{\text{Final weight (g)}}{\text{Initial weight (g)}} \right)$$

Mortality data were analyzed for significant differences ($p = .05$) by Chi-squared contingency tables (Sokal and Rohlf 1995). Initial and final height and weight data were analyzed for signifi-

cant differences using ANOVA (Sokal and Rohlf 1995) after homogeneity of variance was confirmed using Cochran's test (Winer 1991). Size data were \log_{10} transformed where necessary. Where several grades were present at the end of the experiment, a random subsample (the size of which was determined by the proportions in each grade) was used for ANOVA.

RESULTS

Induction of Triploidy

The mean triploidy reading on day 0 was $79.9 \pm .92\%$ (mean \pm SE; $n = 3$ groups of 60 larvae) which increased to 87.8% by 10 weeks' post-settlement. Flow cytometry of gill tissue biopsies from oysters from three farms (Hastings River, Karuah River, and Clyde River) in January/February 1996 gave a mean triploid level of $88 \pm 4.0\%$ (mean \pm SE, $n = 3$ groups of 183–292 oysters).

Larval and Spat Rearing

Poor water quality during the larval run resulted in low survival to settlement (7.5% diploids and 2.0% triploids) with set rates of 56.3% for diploids and 43.2% for triploids. Spat in upweller systems were affected by "post-settlement mortality" (Frankish et al. 1991) in April 1994. Survival from settlement in early March 1994 to May 1994 was 26% for diploid and 31% for triploid spat, respectively. As spat were graded over the same mesh but not matched for size before being supplied to farmers (to avoid matching slow-growing triploids with fast diploids), initial sizes of diploids and triploids were sometimes different. However, this generally favored the diploids with initially larger diploids ($p < .05$) at

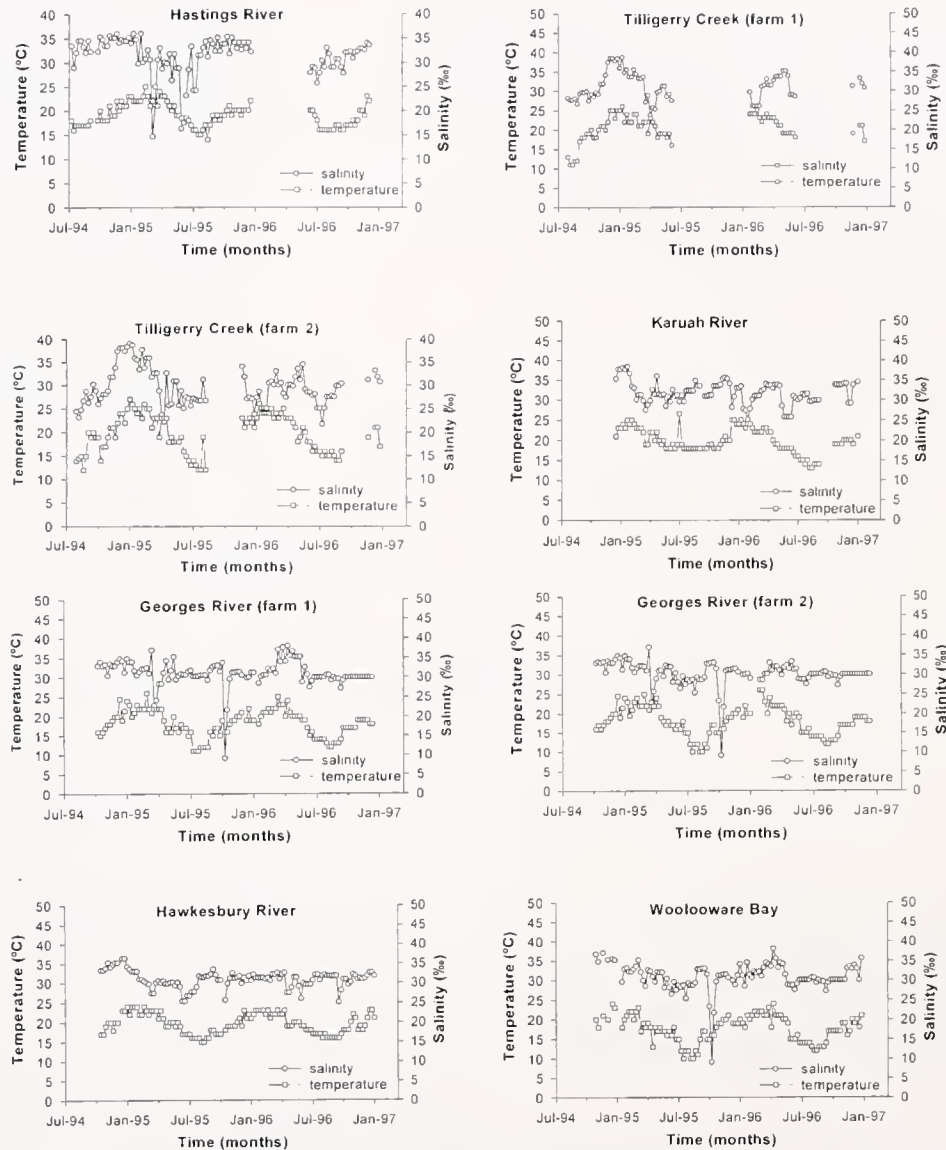


Figure 4. Surface water salinity and temperature data for oyster farms in NSW from July 1994 to December 1996.

8 of the 13 farms, larger triploids at two farms (Hastings River and Tilligerry Creek, 1), and no significant difference ($p > .05$) between the initial size of diploids and triploids at Tilligerry Creek, 2, Brisbane Waters and Shoalhaven.

Appearance

A brown to grey color appearing as distinct patches on the gonad was noted on triploid oysters. This coloration developed during the second year on leases and was most noticeable during summer months.

Whole Oyster Weight

Whole oyster weights over the 2½ year study are shown in Figure 2. For all farms except Lake Merimbula, mean triploid whole weight was greater than mean diploid weight from the time when oysters reached a whole weight of about 5–10 g. Triploids generally remained heavier until the end of the study. Average time taken to reach a mean whole weight of 10 g was 13 months

for triploids and 14 months for diploids (Table 2). Largest increases in mean whole oyster weight occurred during spring through to autumn for northern and central NSW estuaries (Hastings down to Shoalhaven Rivers). The growth season for oysters on the south coast occurred later, i.e., summer through to autumn/early winter. Greatest relative growth of triploids compared with diploids generally occurred later in the growth season after the first year on leases. By the end of the 2½ year study the mean whole oyster weight from the 13 farms involved in the study was 28.3 g (range 18.7–37.4 g) for diploids and 37.0 (20.9–49.4 g) for triploids, a difference of 30.7% (Table 3). Final weights of triploids were significantly greater ($p < .01$) than diploid weights at all 13 farms, with an apparent effect of temperature (Fig. 4) on the relative growth of diploids and triploids. The seven sites with the greatest difference between diploid and triploid growth had mean water temperatures of 18°C or more, and four of the six remaining sites had mean water temperatures of less than 18°C. Time to market size could not be compared between the two ploidy types as diploids had not reached this size by the end of the study;

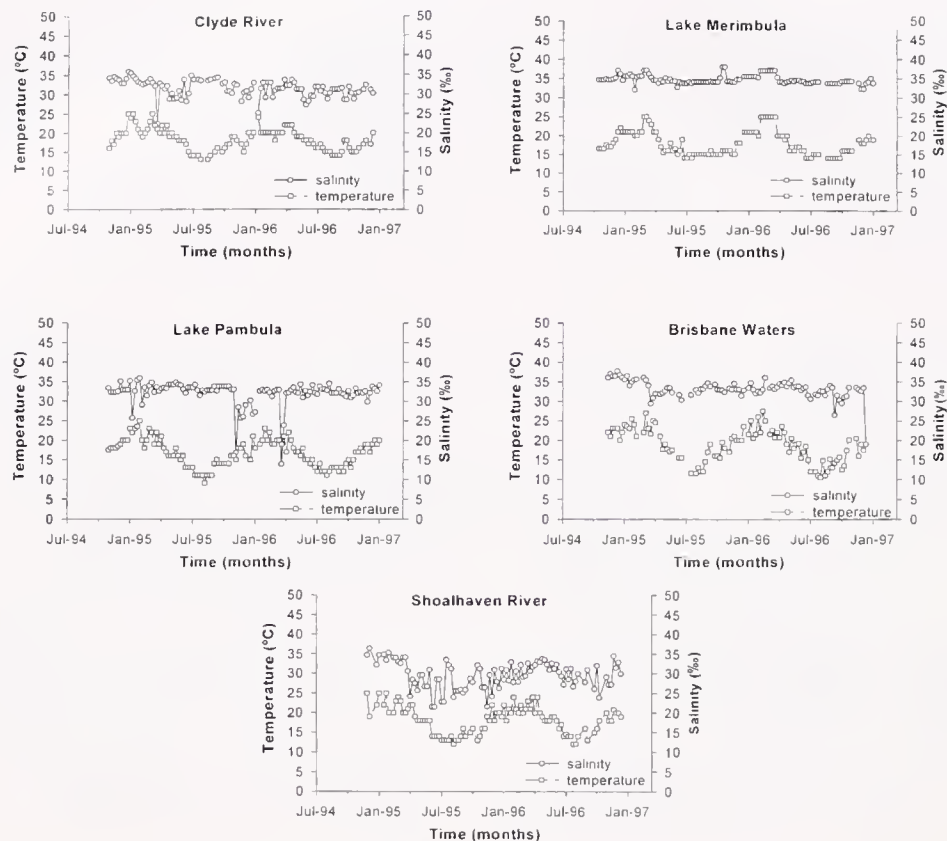


Figure 4. Continued.

instead, the time to reach a mean whole weight of 30 g was compared. Where data are available for both ploidy groups, triploids reached this size 20% faster than diploids. The average time taken for triploids to reach a mean whole weight of 40 g was 26 months for the seven farms that had oysters at this size. There were no farms with diploids at a mean weight of 40 g.

Shell Height

Shell growth of diploids and triploids followed a similar pattern to whole weight (Fig. 3). In general, by the time oysters had reached a mean shell height of 30–40 mm, growth of triploids was greater than that of diploids. Triploids at most farms had reached a shell height of approximately 40 mm, after 8–12 months on leases. Fastest shell growth occurred between winter/spring and autumn for northern and central sites and between spring/summer and autumn for sites south of the Shoalhaven River. Shell growth of triploids relative to diploids was greatest during spring and summer for northern and central sites and between spring and autumn for southern sites. After 2–2½ years on oyster leases, mean shell height was 61.1 mm for diploids and 66.4 mm for triploids, a difference of 8.6% (Table 3) and was significantly larger ($p < .001$) for triploids at 12 of the 13 farms.

Cumulative Mortality

There was little difference between cumulative mortality of diploids and triploids at most sites during the first 12–18 months on leases (Fig. 5). However, cumulative mortality (Table 4) of triploids at the end of the study was significantly ($p < .01$) lower than that of diploids at 6 of the 13 farms and did not differ ($p > .05$)

at 6 of the remaining 7 farms. Cumulative mortality was higher for triploids than diploids at only one site (Tilligerry Creek, farm 1; $p < .05$).

Wild-Caught Diploid: Hatchery Diploid and Triploid Comparison

Initial whole oyster weights and shell heights (Tables 5 and 6) of wild-caught diploids supplied by oyster farmers were significantly ($p < .05$) different from those of hatchery stock for all three sites (data were not homogeneous for the Georges River and Brisbane Waters sites). There was also a significant difference ($p < .05$) between hatchery diploids and triploids at the Georges River site (for both height and weight) and Lake Merimbula (weights). For this reason growth was compared using growth coefficients (Table 7). Wild-caught diploids had lower growth coefficients than both diploid and triploid hatchery stock at all three sites, except for hatchery diploids at Lake Merimbula. High mortality (with symptoms consistent with those of the disease winter mortality) of wild-caught diploids occurred at the Brisbane Waters site during the first year resulting in insufficient numbers to continue the comparison. The cumulative mortality of wild-caught oysters after only 11 months was 55.4% compared with only 6.0 and 2.7% for hatchery diploids and triploids, respectively. For Georges River and Brisbane Waters sites there was a significant effect ($p < .05$) of ploidy type on cumulative mortality (Table 7).

DISCUSSION

The variation in performance of triploid Sydney rock oysters between different sites in the preliminary study of Nell et al. (1994) was emphasized in the present commercial-scale study.

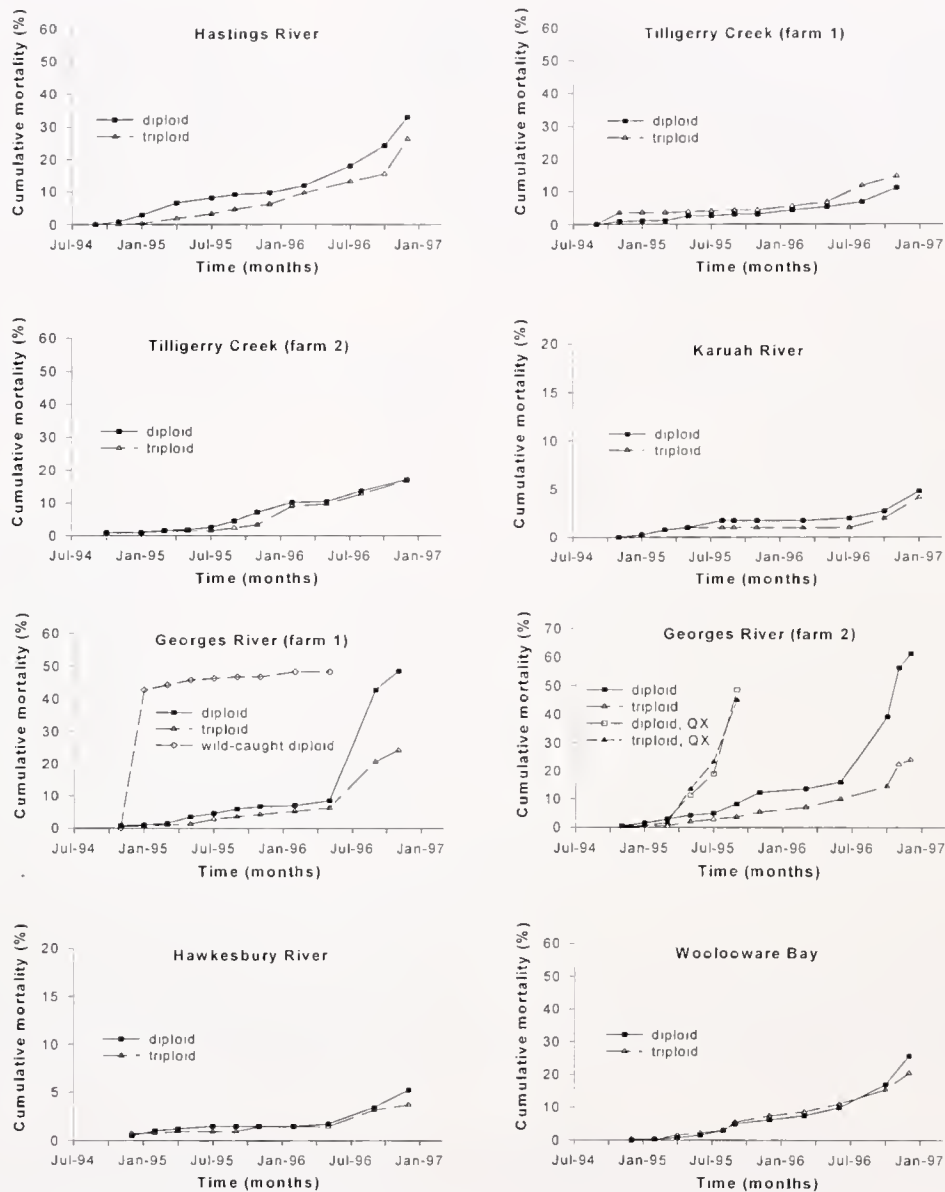


Figure 5. Cumulative mortality (%) of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, grown by commercial oyster farmers in NSW from July 1994 to December 1996.

Much of the variation in this study may be attributable to differences in water temperatures. However, growth of both diploids and triploids at Brisbane Waters, Lake Merimbula, and Hawkesbury River appears to have been influenced by an additional factor, possibly food availability (L. Cooper pers. comm.). Davis (1989b) also measured faster growth rates of triploids relative to diploids at a site with maximum water temperatures (July and August) of 20°C compared with a site with maximum water temperatures of 16°C. This is most likely due to the relatively greater contribution by diploids of energy reserves to gametogenesis at higher temperatures (Shpigiel et al. 1992). The unusually large difference (98.9%) between diploids and triploids at the Hastings River site is partly due to the poor growth of diploids. Oysters at this site were grown subtidally which may account for the faster growth of triploids at this site (Nell et al. 1994). However, growth of diploids appeared to be retarded even before they reached a size at which

we would expect gametogenesis/spawning to affect their growth (Fig. 2).

The growth seasons for both diploids and triploids were consistent with previous studies (Allen and Downing 1986, Nell et al. 1994), i.e., spring through to autumn with greater relative growth of triploids compared to diploids later in the growth season. The period of greater relative growth of triploids occurred prior to the normal spawning season (February to May) for Sydney rock oysters (Nell 1993), i.e., when diploids are diverting a large proportion of their energy stores to gametogenesis at the expense of somatic growth. Triploid Sydney rock oysters did not show an advantage over diploids until they reached a mean whole weight of between 5–10 g or shell height of 30–40 mm. This corresponded to a growout time of between 8 and 14 months and is similar to results (12–13 months) obtained for Pacific oysters in Japan (Akashige and Fushimi 1992) and earlier results (6–9 months) for Sydney

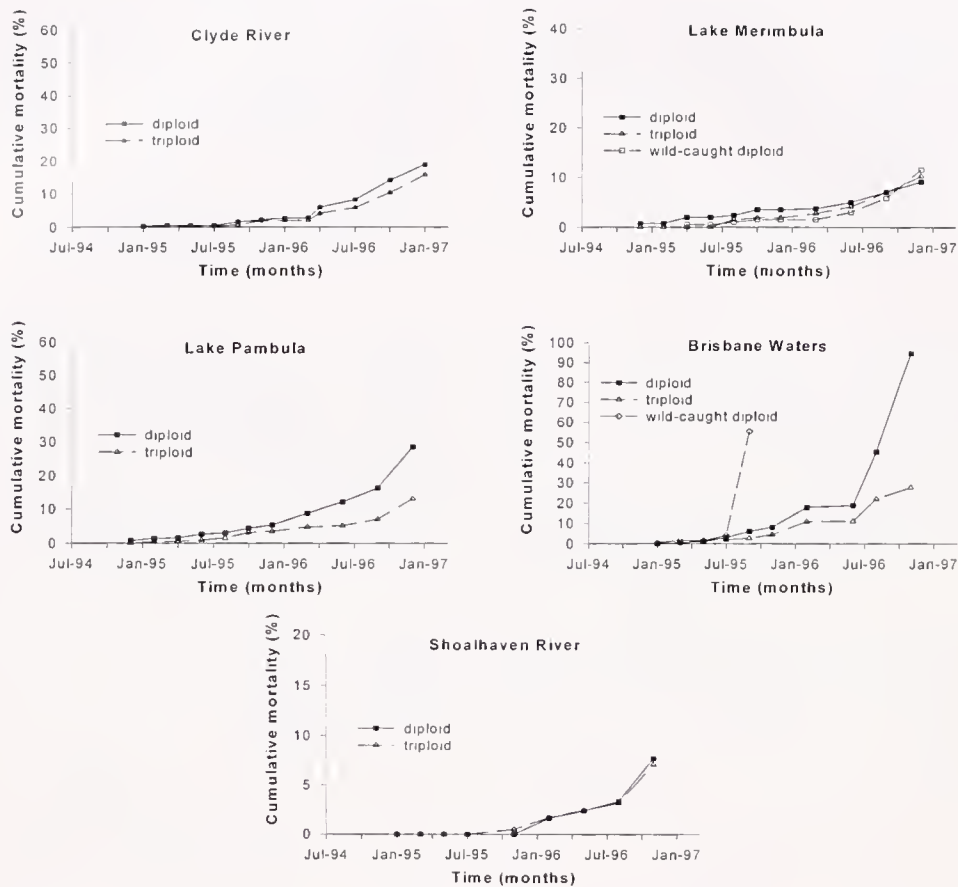


Figure 5. Continued.

rock oysters (Nell et al. 1994). The large time range for the 13 farms emphasizes the fact that benefits of triploids relative to diploids are dependent on size rather than age. This is because the onset of gametogenesis and the ability to spawn in diploid oysters

is related to oyster size rather than age. Singh (1978) found that the American oyster, *Crassostrea virginica* Gmelin, is capable of spawning when it reaches a size of around 25 mm. This is similar to the size (30–40 mm) at which the slower relative growth of

TABLE 4.

Comparison^a of cumulative mortality of diploid and triploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales from July 1994 to November/December 1996.

Estuary	Month Supplied (1994)	Cumulative Mortality (%)		Mean Annual Temperature (°C)
		Diploid	Triploid	
Hastings River ^b	July	32.86	26.22**	19.1
Tilligerry Creek (farm 1)	"	11.06	14.62*	20.4
Tilligerry Creek (farm 2)	August	17.15	16.83	19.7
Karuah River	September	4.80	4.16	20.2
Georges River (farm 1)	"	48.45	24.31***	18.1
Georges River (farm 2) ^c	"	61.11	24.12***	18.0
Hawkesbury River	October	5.26	3.71	19.6
Woollooware Bay	"	25.73	20.48**	17.6
Clyde River ^b	"	18.89	15.76	18.3
Lake Merimbula ^b	"	9.15	10.31	18.0
Lake Pambula ^{b,d}	"	28.60	13.10***	16.7
Brisbane Water ^b	November	94.61	27.66***	18.8
Shoalhaven River	"	7.70	7.09	17.8

*** = $p < .001$; ** = $p < .01$; * = $p < .05$.

^b A weighted mean of more than 1 size grade was calculated for cumulative mortality.

^c Excludes oysters from upriver lease that were removed from the experiment in September 1995 due to low numbers.

^d Oysters divided among three leases.

TABLE 5.

Initial and final^a whole weights^b of diploid, triploid, and wild-caught diploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales.

Estuary	Initial Whole Oyster Weight (g)			Final Whole Oyster Weight (g)		
	Diploid	Triploid	Wild-caught Diploid	Diploid	Triploid	Wild-caught Diploid
Georges River ^c	0.4 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	24.9 ± 0.3	33.8 ± 0.4	30.4 ± 0.5
Lake Merimbula	1.4 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	24.5 ± 0.3	26.4 ± 0.4	24.6 ± 0.3
Brisbane Waters ^c	0.1 ± 0.0	0.1 ± 0.0	0.8 ± 0.0	3.3 ± 0.1	2.0 ± 0.1	9.9 ± 0.5

^a Georges River: November, 1994–May, 1996, Lake Merimbula: April, 1995–December, 1996, Brisbane waters: November, 1994–September, 1995.

^b Mean ± SE (n = 200), a weighted mean of more than 1 size grade was calculated for Brisbane Waters.

^c Comparison was terminated after only 11 months due to high mortality and insufficient numbers of wild-caught diploids.

Sydney rock oyster diploids compared with triploids became apparent in this study.

After 2½ years on commercial oyster leases, triploid Sydney rock oysters were 30.7% heavier (whole oyster weight) and 8.6% larger (shell height) than sibling diploid oysters. In addition, the generally lower growth coefficients of wild-caught diploids compared with hatchery stock at three sites are contrary to the belief of many commercial farmers that hatchery stock is slower growing than wild-caught seed. However, the data for Georges River and Lake Merimbula should be interpreted with caution as wild-caught seed may have been slower growers within a cohort, i.e., they were provided by farmers out of a small size grade from a larger batch of oysters.

There are numerous and conflicting reports on the relative survival of diploids and triploids. The majority suggest similar survival of diploids and triploids post-metamorphosis (e.g., Stanley et al. 1981, Chaiton and Allen 1985, Nell et al. 1994). Davis (1989a) found that, under starvation conditions, diploids survived better than triploids. In contrast, triploid American oysters have been shown to be less susceptible than diploids to the disease MSX, caused by the parasite *Haplosporidium nelsoni* (Matthiessen and Davis 1992) and our present results with Sydney rock oysters illustrate a clear trend of greater triploid survival compared with sibling diploids. This was particularly noticeable at sites affected by the disease winter mortality. Both ploidy types are susceptible to winter mortality (Nell et al. 1994); however, despite identification of winter mortality symptoms (e.g., lesions on the gills and labial palps) fewer triploids died from the disease (Hand et al. 1998). In contrast, Nell et al. (1994) found no significant differ-

ence between mortality of diploids and triploids when grown in Wooloware Bay, NSW, where oysters are prone to winter mortality.

Discoloration of triploid oysters has only been previously reported once when 5.9% of triploid Pacific oysters developed brown discoloration during summer (Maguire et al. 1994b). In Sydney rock oysters, discoloration did not appear to affect the oyster in any other way and was less obvious during the colder months when triploids were readily accepted by oyster processors. As this will most likely be the time when triploids are more marketable, due to their better meat condition, compared with diploids (Nell et al. 1994), it is unlikely to affect their commercialization.

Sydney rock oysters currently take from 3 to 4 years to reach market size (Nell 1993) and may take longer on the colder south coast of NSW. Seven of the 13 farms in this study had marketable triploid Sydney rock oysters (over 40 g mean whole weight) after 2–2½ years on leases whereas there were no farms with diploid oysters at this size. Final mean whole oyster weight of triploids was significantly greater ($p < .01$) than diploid weight at all 13 farms. Labor is one of the major costs of oyster production from spat (Graham 1991). This would be reduced significantly by a reduction in growout time of at least 20%. The better survival of triploid hatchery stock compared with wild-caught diploids and better meat condition during winter compared with diploids would further improve the profitability of farming triploid oysters.

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TABLE 6.

Initial and final^a shell heights^b of diploid, triploid, and wild-caught diploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales.

Estuary	Initial Shell Height (mm)			Final Shell Height (mm)		
	Diploid	Triploid	Wild-caught Diploid	Diploid	Triploid	Wild-caught Diploid
Georges River ^c	15.5 ± 0.1	16.1 ± 0.1	17.9 ± 0.2	61.0 ± 0.3	65.7 ± 0.4	63.8 ± 0.6
Lake Merimbula	24.0 ± 0.3	23.3 ± 0.3	22.5 ± 0.3	56.6 ± 0.4	60.4 ± 0.4	59.2 ± 0.4
Brisbane Waters ^c	9.8 ± 0.1	9.7 ± 0.1	21.5 ± 0.5	25.0 ± 0.4	22.1 ± 0.4	40.9 ± 0.9

^a Georges River: November, 1994–May, 1996, Lake Merimbula: April, 1995–December, 1996, Brisbane Waters: November, 1994–September, 1995.

^b Mean ± SE (n = 200), a weighted mean of more than one size grade was calculated for Brisbane Waters.

^c Comparison was terminated early due to high mortality and insufficient numbers of wild caught diploids.

TABLE 7.

Growth coefficients^a and cumulative mortality of diploid, triploid, and wild-caught diploid Sydney rock oysters *Saccostrea commercialis* grown by commercial oyster farmers in New South Wales.

Estuary ^b	Growth Coefficient (G ₉₀)			Cumulative Mortality (%)		
	Diploid	Triploid	Wild-caught Diploid	Diploid	Triploid	Wild-caught Diploid
Georges River ^c	0.676	0.726	0.617	8.5	6.2	48.2
Lake Merimbula	0.420	0.466	0.431	10.1	8.5	11.6
Brisbane Waters ^{c,d}	1.016	0.899	0.755	6.0	2.7	55.4

$$^a G_{90} = \frac{90}{\text{Duration (days)}} \times \ln\left(\frac{W_t}{W_0}\right)$$

Where: W_t = final mean weight of oysters and W_0 = initial mean weight of oysters.

^b Georges River: November, 1994–May, 1996, Lake Merimbula: April, 1995–December, 1996, Brisbane Waters: November, 1994–September, 1995.

^c Comparison was terminated early due to high mortality and insufficient numbers of wild-caught diploids.

^d A weighted mean of more than one size grade was used for calculation of the growth coefficient.

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STUDIES ON TRIPLOID OYSTERS IN AUSTRALIA. XI. SURVIVAL OF DIPLOID AND TRIPLOID SYDNEY ROCK OYSTERS (*SACCOSTREA COMMERCIALIS* (IREDALE AND ROUGHLEY)) THROUGH OUTBREAKS OF WINTER MORTALITY CAUSED BY *MIKROCYTOS ROUGHLEYI* INFESTATION

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ABSTRACT Diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, were grown at seven sites in New South Wales (NSW) for 25–28 months and exposed to the parasite *Mikrocytos roughleyi* over two consecutive winters during the period August–November 1994 to December 1996. Triploids showed a higher survival rate than diploids. Over the second winter/spring, average cumulative mortality of diploids across all sites, was 35.0% (range 6.7–76.8%) compared with only 12.2% (range 4.0–18.1%) for triploids. This reduction in mortality during the second year of culture on leases, combined with the growth and condition advantages that triploidy confers, could provide the Sydney rock oyster industry in NSW with significant improvements in profitability.

KEY WORDS: Disease, oysters, triploid, winter mortality

INTRODUCTION

Production by the oyster farming industry in New South Wales (NSW), Australia is irregularly affected by three principal diseases: winter mortality caused by the protistan parasite, *Mikrocytos roughleyi* (Farley et al. 1988), QX caused by the haplosporidian parasite, *Marteilia sydneyi* (Perkins and Wolf 1976), and mudworms, the most harmful of which is *Polydora websteri* (Skeel 1979).

Winter mortality can cause losses of up to 80% of a crop of market size oysters (Wolf 1967) with most mortality occurring from late winter to spring. The disease is limited to the mid- to southern part of the range in which *S. commercialis* is farmed (Nell 1993), corresponding to its greater prevalence in colder waters (Roughley 1926). In addition, cold autumn/winter temperatures following dry autumn weather (resulting in high estuarine salinities) are generally associated with an increase in the severity of infestation by *M. roughleyi* (Wolf 1967). This may account for the frequently observed association between oysters with high meat condition and those affected by winter mortality as spawning is generally stimulated in *S. commercialis* by high temperatures and a reduction in salinity (Nell and Smith 1988). Alternatively, meat condition may have a more direct effect on the incidence of winter mortality.

M. roughleyi infestation of oysters (winter mortality) is characterized by the appearance of yellow to brown lesions on the labial palps, mantle, gills, gonad, and/or adductor muscle (Roughley 1926, Wolf 1967). Oysters eventually lose the ability to remain closed when exposed to air at low tide. Despite the identification of the species responsible for winter mortality (Farley et al. 1988), the mechanisms by which *M. roughleyi* infests Sydney rock oysters and its physiological effects are still poorly understood.

Mortality has frequently been compared between diploid and triploid bivalves with varying results (e.g., Barber and Mann 1991,

Meyers et al. 1991, Mathiessen and Davis 1992). Several authors have proposed that any enhanced performance of triploids over diploid oysters results from 'hybrid vigor' derived from their greater heterozygosity (e.g., Beaumont and Fairbrother 1991, Hawkins et al. 1994). However, these authors were referring to meiosis I triploids which are generally (depending on the recombination rate) more heterozygous than both diploids and meiosis II triploids (Guo et al. 1992b, Hawkins et al. 1994). In fact, a comparison of mortalities between meiosis I and meiosis II triploids, and that of diploid oysters frequently illustrates the opposite trend, i.e., higher mortality of meiosis I triploid bivalves as embryos or during growout compared with both meiosis II triploids and diploids (Stanley et al. 1984, Beaumont and Kelly 1989, Guo et al. 1992a). Consequently, the greater heterozygosity of meiosis II triploids compared with diploids would not be expected to influence their relative mortality rates.

In an earlier study in Woollooware Bay, NSW (Nell et al. 1994), a comparison of mature diploid and triploid Sydney rock oysters showed no significant difference in mortality (45.9% of triploids and 41.3% of diploids) over a winter/spring season (May to December 1992). Oysters in Woollooware Bay are frequently affected by winter mortality and the experimental oysters displayed signs typical of the disease. On the basis of that experiment, a difference in susceptibility of diploids and triploids to winter mortality would not be expected. A commercial farming trial of diploids and triploids conducted throughout NSW at 13 oyster farms between 1994 and 1996 revealed, however, a greater overall rate of mortality of diploids compared with triploids (Hand et al. 1998). Data for sibling diploid and triploid oysters are presented here from the seven commercial oyster leases in four NSW estuaries where winter mortality was observed during this study.

METHODS

Production of Spat

The methods of triploidy induction along with those for rearing the larvae and spat are described in the associated commercial

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farming study on triploid and diploid Sydney rock oysters in NSW (Hand et al. 1998).

Oyster Management

Oysters used in this study formed part of a larger commercial scale farming trial with diploid and triploid Sydney rock oysters on 13 oyster farms distributed along the NSW coast. Spat (25,000) of each ploidy were supplied to participating farmers between August and November 1994 and monitored regularly for growth and mortality until December 1996. Mortality data were collected for all sites (Hand et al. 1998) but only the data from seven sites from four estuaries [Lake Pambula (sites 1, 2, and 3), Woollooware Bay (Georges River farms 1 and 2), Brisbane Waters, and Tilligerry Creek (farm 2)] that were identified as having oysters with winter mortality during the course of the study are presented here.

Oysters in these four estuaries suffer regularly from winter mortality being in the mid- to southern half of the state. Winter mortality in the oysters was diagnosed from the appearance of yellow to brown lesions on the labial palps, gills, mantle, adductor muscle, and/or gonad (Roughley 1926). All participating farmers grew the diploid and triploid oysters using the same standard commercial methods; stocking densities were standardized within estuaries (Hand et al. 1998). As this study was based on commercial farming methods, oysters were graded at all sites when large size ranges developed to allow "normal" growth of small oysters by removing competition from larger oysters.

Initially, oysters from Georges River, farm 2, were divided between two leases in the same bay. As there was little difference in growth and mortality of oysters between the two leases after 12 months, these oysters were recombined. Oysters at Lake Pambula were divided among three farmers (sites 1–3) when they reached a large enough volume (after 6 months at site 3).

As is commercial practice (Nell et al. 1988), oysters were moved where possible during winter to avoid infestation by *M. roughleyi*. Oysters from both Georges River farms were moved to up-river leases from May/June through to November/December during the second winter (1996). Tilligerry Creek oysters were moved up-river in August and were returned in November during the first winter (1995) and August to December during the second winter (1996).

An additional comparison to the hatchery diploids and triploids was possible at the Brisbane Waters' site with wild-caught diploids (provided by the farmer) grown under the same conditions as hatchery stock.

Sampling Strategy

Being a commercial-scale study, the large number of oysters at each site (50,000 in total) made sampling of all stock logistically difficult. For this reason, mortality was assessed from a random 10% (by volume) of stock every 2 months until November 1995 when the sampling frequency was changed to once every 3 months. Where several grades were present, a weighted mean of mortality was determined (see Statistical Analyses). Oyster shells from dead oysters were returned to trays/baskets after each sampling episode (unless all stock was brought in for sampling) to maintain equivalent apparent mortality between sampled stock and stock remaining on leases. Total weight for both diploid and triploid groups was measured every 2 months for the first 6 to 9 months of the study to assess overall losses of stock as well as proportions in each grade. As handling became more difficult with

the increase in volume of stock, farmers were given the choice of bringing in either a random 25% or all stock every 6 months for measurement of total weight. Weights of dead oysters and over-catch were weighed and accounted for when total weights were measured.

Temperature and salinity were determined weekly by farmers using a thermometer and hydrometer. Salinity values were calculated using a density/temperature conversion table (Wolf and Collins 1979).

Statistical Analyses

Monthly percent mortality is presented as a weighted mean when oysters were in several grades:

$$\bar{X}_w = \frac{\sum_{i=1}^n W_i \bar{X}_i}{\sum_{i=1}^n W_i}$$

where \bar{X}_w is the weighted mean of mortality calculated from the mean \bar{X}_i of each of n grades, and each \bar{X}_i weighted by a factor W_i (Sokal and Rohlf 1995). The total number in each grade was used as a weighting factor and was estimated from the total weight of all oysters for each grade divided by the mean weight of individual oysters for that grade (Hand et al. 1998). Cumulative percent mortality was calculated for the period June–December for both years to determine the effects of winter mortality, which are generally expressed from June to October (Nell and Smith 1988) (as sampling was 3 monthly, November and December were included). Monthly mortality (frequency dead/alive) and cumulative mortality were analyzed for association with ploidy level using Chi-squared contingency tables (Sokal and Rohlf 1995).

Initial shell heights of hatchery diploids and triploids and wild-caught diploids were compared using analysis of variance (Sokal and Rohlf 1995) after homoscedasticity was confirmed with Cochran's test (Winer et al. 1991).

RESULTS

For all seven sites, signs consistent with the disease winter mortality (see *Oyster Management*) were found in both diploid and triploid oysters. Salinity and temperature data are shown in Figure 3.

Tilligerry Creek

Winter mortality signs were noted in diploids and triploids from Tilligerry Creek in August 1995 but mortality was low (<2%) for both ploidy types (Fig. 1) with no significant effect of ploidy on mortality ($p > .05$). However, there was a significant association between ploidy and cumulative mortality over the winter/spring of 1995, with higher mortality of diploids compared with triploids (Table 1). Oysters were moved in August of both years to "over-winter" at a site in Port Stephens (Tea Gardens) where they have previously been less prone to the disease (unfortunately, salinity and temperature data are unavailable for the Tea Gardens site). Although some mortality occurred (Fig. 1), winter mortality signs were not evident during the winter/spring of 1996. Mean temperatures and salinities (where data are available) indicate generally cooler temperatures (13.8°C c.f. 15.5°C) and similar salinities (27.3‰ c.f. 27.2‰) for the winter of 1995 compared to 1996.

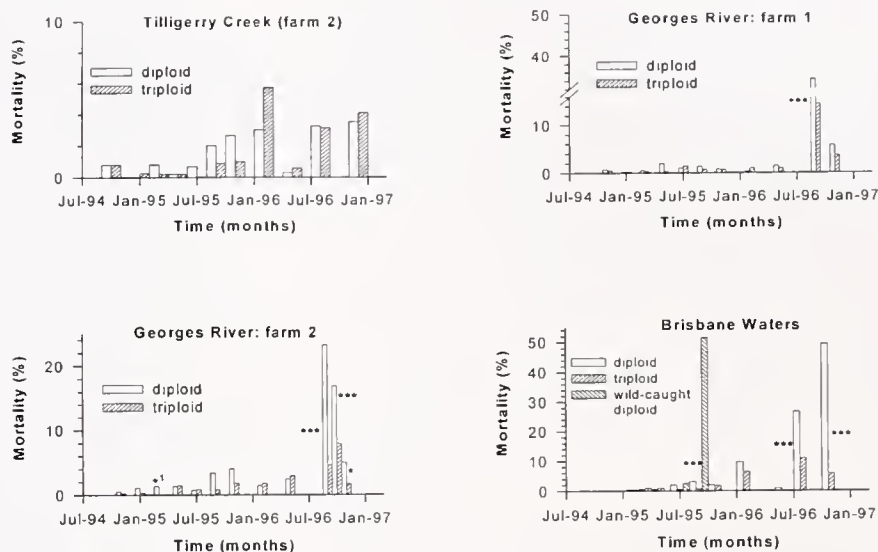


Figure 1. Monthly mortality (%) of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, grown by commercial oyster farmers in NSW (* = $p < .05$; ** = $p < .01$; *** = $p < .001$; ¹interpret with caution as frequency < 5 in more than 20% of contingency table cells).

Brisbane Waters

When compared with hatchery diploids and triploids, wild-caught diploids were found to have significantly ($p < .05$) larger initial shell heights (21.5 mm compared with 9.8 and 9.7 mm for diploids and triploids, respectively, $n = 400$; data were heterogeneous). Wild-caught diploids suffered significantly higher mortality ($p < .001$), associated with winter mortality signs, than hatchery diploids and triploids during the winter of 1995, with a mortality rate of 51.5% for the period August–September compared with 3.1 and 0.7%, respectively (Fig. 1). Insufficient numbers of wild-caught diploids remained after the first year to continue the comparison. Mortality of hatchery oysters, associated with winter mortality signs in oysters at the Brisbane Waters site, was high during both the 1995 and 1996 winter/spring seasons (Fig. 1). There was a highly significant effect of ploidy level ($p < .05$) on cumulative mortality between June–September, 1995 and 1996 (Table 1). Monthly and cumulative percent mortality rates of diploids during

winter/spring were consistently higher than that of triploids. For example, cumulative mortality of diploids during the 1996 winter/spring period was 76.8% compared with only 16.9% for triploids. Mean salinity over winter at this site was lower (26.6‰) in 1995 than 1996 (29.2‰) and temperature was slightly higher for the 1996 winter season (14.2°C c.f. 13.6°C).

Georges River: Farm 1

No winter mortality signs were noted in either diploid or triploid oysters during 1995, with no significant effect of ploidy level on cumulative mortality. Winter mortality was noted in diploids and triploids in September 1996 (despite having been moved up-river in May) with a highly significant ($p < .001$) effect of ploidy on cumulative mortality (40.0 and 18.1% for diploids and triploids, respectively) (Table 1). Both mean temperature and salinity were slightly lower during the 1996 winter compared with the 1995

TABLE 1.

Cumulative mortality^a of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, over two consecutive winter/spring seasons in estuaries where the disease winter mortality occurs.

Farm	June–December, 1995—Cumulative Mortality (%)				June–December, 1996—Cumulative Mortality (%)		
	Diploid	Triploid	Wild-caught Diploid ^b	Ploidy Effect ^d	Diploid	Triploid	Ploidy Effect ^d
Tilligerry Creek (farm 2) ^c	5.3	1.9	—	*	6.7	7.2	n.s.
Georges River (farm 1) ^c	3.2	3.0	—	n.s. ^e	40.0	18.1	***
Georges River (farm 2) ^c	8.1	3.4	—	**	45.0	14.2	***
Lake Pambula (site 1)	4.4	3.3	—	n.s. ^e	49.9	16.4	***
Lake Pambula (site 2)	1.6	1.0	—	n.s. ^e	11.1	4.0	**
Lake Pambula (site 3)	2.5	1.6	—	n.s. ^e	15.2	8.3	**
Brisbane Waters	7.0	2.9	53.9	***	76.8	16.9	***

^a Cumulative % mortality for the period June to December for both years.

^b Spat provided by farmer had a larger initial size than hatchery stock; discontinued in first year due to insufficient numbers.

^c Stock at Tilligerry Creek and both Georges River farms was moved upriver during winter.

^d Chi² test of association of ploidy and number dead/alive: n.s. = $p > .05$; * = $p < .05$; ** = $p < .01$; *** = $p < .001$.

^e Frequency was less than 5 in $>20\%$ of 2×2 contingency table cells so results should be interpreted with caution.

winter at this site at 13.9°C and 31.0‰ c.f. 13.6° and 29.5‰ for 1995 and 1996, respectively.

Georges River: Farm 2

Winter mortality signs were evident in both diploids and triploids during both 1995 and 1996 winter/spring seasons, with generally higher mortality in diploids than triploids (Fig. 1). This was particularly evident during the second winter (1996) with cumulative mortality of 45.0% of diploids compared with only 14.2% of triploids (Table 1). Ploidy level had a significant ($p < .01$) effect on cumulative mortality over winter/spring for both years (Table 1). There was little difference between mean salinity over winter 1995 (29.2‰) and 1996 (29.5‰); similarly, mean temperature over winter in 1995 was 13.2°C compared with 13.6°C over the 1996 winter season.

Lake Pambula: Sites 1–3

Winter mortality signs were observed during the second winter corresponding to an increase in monthly mortality in both diploids and triploids at all three sites (Fig. 2). The effect on cumulative mortality was greatest at site 1 with 49.9% of diploids and 16.4% of triploids dying between June and December 1996. For all three sites there was a significant association ($p < .01$) between ploidy level and mortality with a higher cumulative mortality of diploids occurring in all cases (Table 1). A small increase in mortality of diploids was noted in March 1996 at site 3 but not at the other two sites. Mean winter temperatures at Lake Pambula were lower than the more northern sites for both 1995 and 1996 (11.9 and 12.8°C, respectively). Salinity was high compared with other sites and similar for both winter seasons (32.9 and 32.8‰).

DISCUSSION

The high mortality of wild-caught diploids at the Brisbane Waters site during the first winter season (1995) compared with relatively low hatchery diploid and triploid mortality, may be attributed to the greater mean size of wild-caught oysters. Larger oysters

are known to be more susceptible to winter mortality (Wolf 1967). The difference in susceptibility of diploids and triploids to winter mortality was most pronounced at this site, and greater in the 1996 than in the 1995 winter. Despite the cumulative mortality of triploids (16.9%) being similar to that of triploids at the other sites (mean: 12.2%; range 4.0–18.1%), visual inspection of a sample of hatchery diploids at this site showed they were severely affected by the parasite (76.8% cumulative mortality). Oysters at Brisbane Waters were not moved over winter to avoid infestation (in contrast to oysters at both Georges River sites and Tilligerry Creek), accounting for the higher mortality of diploids. However, this does not explain the relatively low mortality of triploids at Brisbane Waters. A similar trend of greater triploid survival relative to diploids through outbreaks of winter mortality was found for all sites: average cumulative mortality over winter/spring, 1996 of diploids across all seven sites was 35.0% (range 6.7–76.8%) compared with only 12.2% for triploids (range 4.0–18.1%) despite farmers at three of the sites using standard disease management strategies (i.e., moving stock during winter/spring). Average mortality of triploids at the eight unaffected sites in the associated farming study was 6.1% during the same winter/spring period and was 7.0% for diploids. Triploids appear to have some inherent property that enables them to cope better than diploids with infestation by *M. roughleyi*. However, triploids are not resistant to infestation by *M. roughleyi* and do not always survive outbreaks of the disease better than diploids. Nell et al. (1994) compared diploid and triploid Sydney rock oysters at a site in Woolloomare Bay, NSW and found no significant difference in mortality between May and December (winter–spring). Similarly, although data are not available, high mortality with signs of *M. roughleyi* infestation of both diploid and triploid Sydney rock oysters was observed at a site in Lake Merimbula, NSW over winter 1996 (P. Maguire pers. obs., oyster farmer, 1996).

Cold winter temperatures and high salinity are the main factors associated with increased mortality from *M. roughleyi* infestation (Wolf 1967) and this is generally supported by the conditions prior to and during outbreaks in the present study. Most mortality oc-

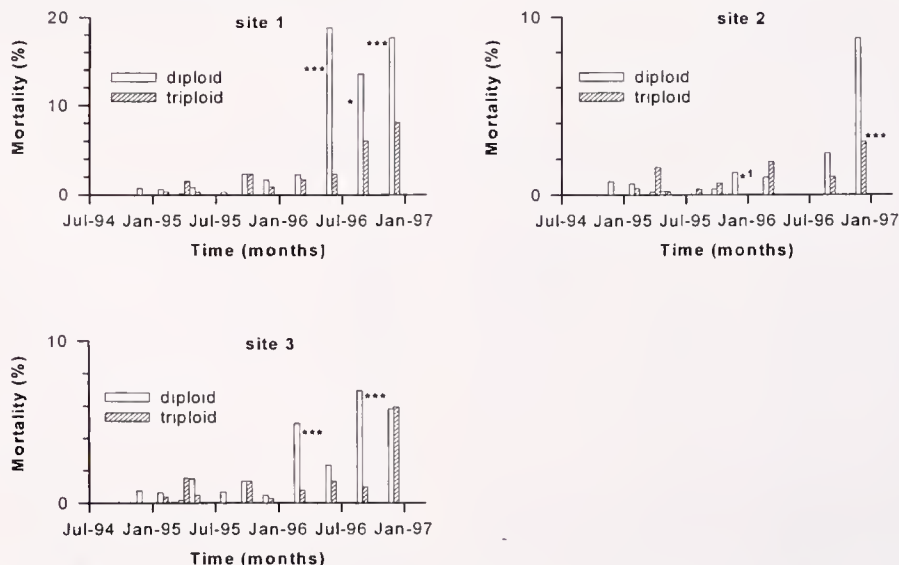


Figure 2. Monthly mortality (%) of diploid and triploid Sydney rock oysters, *Saccostrea commercialis*, grown by commercial oyster farmers in Lake Pambula, NSW (* = $p < .05$; ** = $p < .01$; *** = $p < .001$; 'interpret with caution as frequency < 5 in more than 20% of contingency table cells).

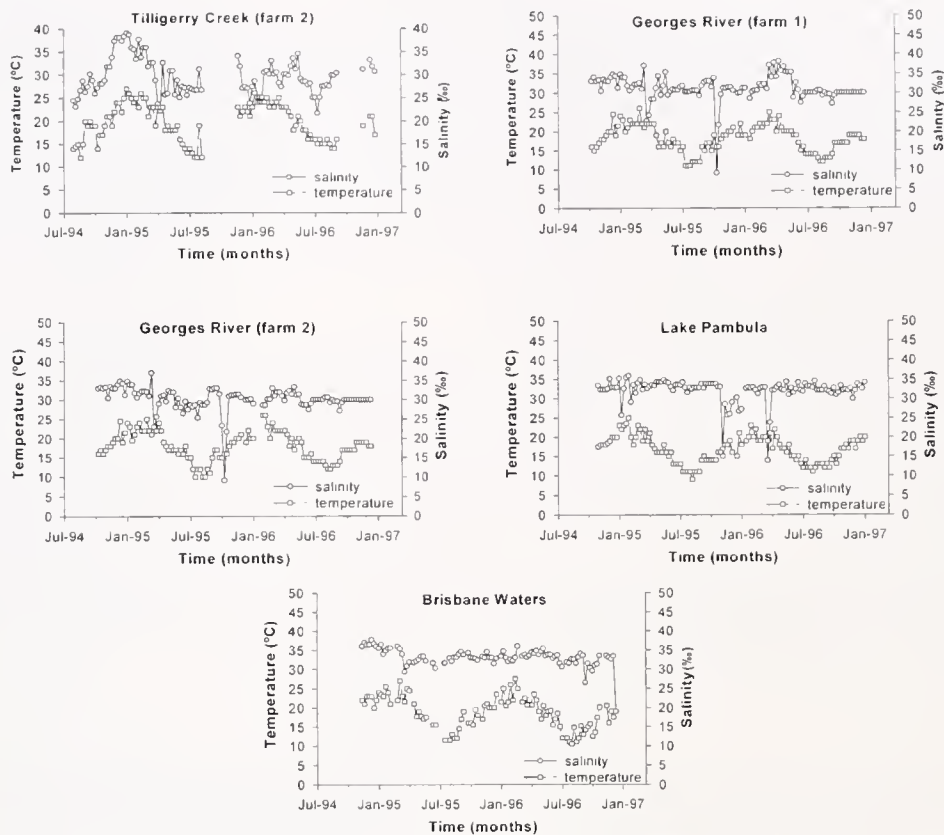


Figure 3. Surface water salinity and temperature data for oyster farms in NSW from August 1994 to December, 1996.

curred when mean winter water temperatures were 14°C or less and mean salinity 29‰ or higher. Physical conditions do not, however, explain the particularly severe outbreak of winter mortality at Brisbane Waters in 1996. Conditions of temperature and salinity at the Brisbane Waters site in 1996 were not particularly unfavorable compared with the remaining sites, yet 77% of hatchery diploids died (c.f. 17% of triploids).

Winter mortality generally affects the larger oysters in a population (Wolf 1967). This may simply be due to the greater volume of water filtered by larger oysters, increasing the likelihood of being exposed to the parasite, or a cumulative effect of parasite exposure in older/larger oysters. Many farmers also believe that oysters with high meat condition are more susceptible to the disease. The increased survival of triploids compared with diploids is therefore contradictory, as triploids in the associated farming study (Hand et al. 1998) were on average 30% heavier and generally had better meat condition over winter than sibling diploids (R. E. Hand unpublished data). A comparison of mortality and growth data suggests a size-threshold for both diploids and triploids above which they are more susceptible to the disease. That is, when exposed to *M. roughleyi*, mortality seemed to be greater in oysters at or above a mean size of 40 mm shell height and 10 g whole oyster weight. Although this is an approximate size only and smaller oysters are still affected (to a lesser extent) by the disease, it is similar to the size at which triploid Sydney rock oysters show a growth advantage over diploids (Hand et al. in press); that is, when diploids are diverting a greater proportion of their energy to gametogenesis. This may indicate a relationship between the state of gonad development (or gonad composition) and the effect of winter mortality on oysters and is supported by the fact that diploid

oysters of better meat condition are more susceptible to the disease. As triploids had a higher meat condition than diploids over winter it is likely that the changes in gonad composition of diploids with increasing size (as opposed to meat condition) is influencing susceptibility to the disease. That is, the different composition of the triploid gonad, due to limited gamete development (and similar to the immature diploid), may either confer an advantage to the oyster when challenged by the disease or discourage initial infestation.

The protistan parasite *Perkinsus marinus* causes the disease 'Dermo' in American oysters *Crassostrea virginica*. The proportions of phospholipids and specific fatty acids in *P. marinus*, compared with the host oyster indicate possible active assimilation of fatty acids from the oyster (Volety et al. 1995). *P. marinus* has also been shown to cause a reduction of up to 40% of free amino acid levels in oysters (Paynter 1996) which disturbs the animal's osmoregulatory capacity. Fatty acids, lipids, and amino acids constitute important energy reserves to animals during periods of low food availability (Stryer 1988) such as are experienced in winter in NSW (Roughley 1926). *M. roughleyi* infested oysters are often characterized by an inability to maintain valve closure when out of water. This has usually been attributed to the appearance of lesions in the adductor muscle (Roughley 1926). The same symptom in *P. marinus* infested oysters has been attributed to lower glycogen levels in the adductor muscle of infested oysters (Dwyer and Burnett 1996). Glycogen levels in diploid oysters generally fluctuate throughout the year with a similar pattern to condition index. Levels over winter are generally low following spawning in summer/spring; in contrast, triploids maintain relatively stable glycogen levels throughout the year (Nell et al. 1994). Smith (1991) found

no difference in glycogen levels in *S. commercialis* with varying degrees of winter mortality although a general decline occurred over winter and glycogen was measured on a whole meat basis rather than in individual tissues such as the adductor muscle. *M. roughleyi* may have an effect similar to *P. marinus* of reducing localized energy stores when whole body levels of glycogen are generally low. In addition, a disturbance of free fatty acid and amino acid levels during winter (when lipids and amino acids are important sources of energy) would have serious physiological consequences which triploids may be able to avoid, having higher glycogen stores available. Infestation may also be related to initial free fatty acid levels which would be higher in overmature diploids. That is, oysters that retain developed gametes for an abnormally long period suffer changes in lipid metabolism leading to abnormally high levels of free fatty acids in the gonad (Mori 1979). Oysters are more likely to retain gametes through to winter during years of cold winter temperatures and low rainfall (conditions that are also known to increase the incidence of winter mortality) (Nell and Smith 1988).

In conclusion, although triploid Sydney rock oysters do not always survive *M. roughleyi* infestation better than diploids, in the present study triploidy had a significant ($p < .01$) effect on mortality at six of the seven sites where winter mortality occurred

during the final year on leases. If these results could be applied on a broad scale to estuaries where winter mortality accounts for a major proportion of stock losses and costs, farming of triploid Sydney rock oysters could substantially improve the profitability of commercial oyster farming in NSW. That is, farming triploid Sydney rock oysters using standard disease management practices would, on average, reduce the loss of stock to winter mortality by 65%, in the final year on leases (when oysters are most valuable as well as normally being more susceptible to the disease).

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CHANGES IN BENTHIC MACROFAUNA ASSOCIATED WITH INTERTIDAL OYSTER, *CRASSOSTREA GIGAS* (THUNBERG) CULTURE

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ABSTRACT Changes in community structure of an intertidal, macrobenthic community in the vicinity of a *Crassostrea gigas* (Thunberg) cultivation site were studied. Samples were taken underneath trestles, in access lanes and at a control site. No evidence of organic enrichment was found, linked to the highly dissipative nature of the study site, the latter as evidenced by the granulometric composition of the substrate and the species encountered. Compared to the community at a control site, the macrofauna in the access lanes has undergone species displacements and changes in numerical abundance of surficial living forms and shallow, fragile burrowers. This is postulated to be linked to physical disturbance by heavy vehicle traffic.

KEY WORDS: Macrofauna, oyster cultivation, *Crassostrea gigas*, impact

INTRODUCTION

Over the last few decades a considerable amount of scientific literature has been devoted to the environmental impacts associated with coastal aquaculture. This has been primarily driven by the increasing importance of aquaculture as a coastal resource and potential conflicts arising from perceived negative impacts of suspended finfish culture. These usually take the form of modifications in the soft-sediment community structure in the vicinity of the aquaculture sites (Gowen and Bradbury 1987, Henderson and Ross 1995) or interactions of chemicals used on the farms with the surrounding ecosystem (Pillay 1992).

Although on a world-wide scale bivalve aquaculture is perhaps of less economic importance than finfish culture, in some countries considerable areas of coastline are dedicated to either mid-water mussel culture or intertidal oyster and clam culture. In terms of benthic community modifications, some of the environmental effects of suspended bivalve culture are similar to those described for finfish farming (Mattson and Linden 1983). In contrast, most studies on intertidal culture have either failed to demonstrate any significant changes in benthic community structure (Mojica and Nelson 1993) or have only detected minor changes (Nugues et al. 1996, Kaiser et al. 1996, Spencer et al. 1996). Nevertheless, in view of the large spatial scale of many intertidal bivalve aquaculture operations, the potential exists to induce large-scale changes (Nugues et al. 1996), in contrast to the usual smaller spatial scale of finfish operations. In the USA, acute disturbances, such as the manipulation of burrowing decapods with pesticide treatments or mechanical harvesting in intertidal bivalve culture (Simenstad and Fresh 1995) are thought to strongly influence ecosystem carrying capacity. These potential habitat and community changes are considered important in the UK, Canada, and Ireland in view of the often close proximity between bivalve mariculture operations and sites of nature conservation interest, particularly in relation to birds (Dickson et al. 1990).

Very few studies have been carried out on benthic community changes associated with intertidal oyster (*Crassostrea gigas*) culture. Castel et al. (1989) provide summarized information on total abundance and biomass changes at two intertidal sites in Arcachon, France, one site having trestle-type cultivation and the other

having 'parc' culture on the seabed. Nugues et al. (1996) studied benthic community changes in more detail at one site (trestle-type cultivation) in the River Exe, UK. Summarized information on environmental impacts of large-scale bottom-type cultivation in Washington and Oregon (USA) is found in Simenstad and Fresh (1995). In contrast, biodeposition from pseudofecal and fecal production by *C. gigas* and the resulting chemical changes in both the sediment and overlying water column have been extensively studied (Deslous-Paoli et al. 1987, 1992, Sornin et al. 1983).

The aims of the present contribution were to assess potential changes in macrofaunal community structure underneath trestles and in access lanes associated with large scale oyster culture in Dungarvan Bay.

METHODS

The present study was carried out during September 1995 in Dungarvan Bay, SE Ireland, the second largest oyster cultivation area in Ireland, producing about 500 tonnes of *C. gigas* per year. Oyster cultivation is carried out in the mid- to low intertidal region of the bay, with all growing sites adjacent to each other along the southern shoreline. The actual studied cultivation area was the most northeastern site (approx. area 12.5 hectares), and had been established as a growing site for 6 years prior to sampling. Oysters are grown in poches on trestles fastened onto metal frames. Twinned rows of trestles are positioned perpendicular to the water line, with access corridors in between each twinned set. The production of the site is approximately 200 tonnes of seed oysters per annum.

Two different treatments were sampled, corresponding to different potential impacts: (1) directly underneath oyster trestles, potentially subjected to additional organic input from oyster fecal or pseudofecal material (designated as Trestle); and (2) in access corridors, potentially subjected to compaction of sediment due to the use of heavy vehicles (designated as Lane). In addition, a Control site was established 300 m away from the edge of the cultivation area. All sampling sites were situated approximately at midtide level.

Ten samples were obtained from each treatment, with samples randomly positioned within each treatment area. Samples for faunal analysis were obtained by manually removing the substratum

TABLE 1.
Sedimentary characteristics of treatments.

	Lane	Trestle	Control	F _{2,27}
% Gravel	1.631 (0.365)	1.442 (0.239)	1.986 (0.423)	0.619 ^{ns}
% Coarse sand	1.349 ^a (0.264)	0.894 ^b (0.067)	2.519 ^{a,b} (0.351)	10.650*
% Medium sand	5.415 ^a (0.685)	4.005 ^b (0.261)	9.300 ^{a,b} (0.990)	14.404*
% Fine sand	91.460 ^a (1.105)	93.486 ^b (0.382)	85.833 ^{a,b} (1.367)	14.586*
% Silt-clay	0.144 (0.008)	0.173 (0.002)	0.363 (0.145)	1.975 ^{ns}
% Organic matter	0.963 (0.007)	0.964 (0.007)	0.967 (0.006)	0.138 ^{ns}

Mean values and standard errors are given, together with results from a one-way ANOVA test on arcsin transformed data.

* $p < .0001$ or ^{ns} nonsignificant. Values sharing the same superscript are significantly different (Bonferroni post-hoc comparison, $p < .05$).

within a 0.1 m² square frame which was pushed into the sediment to a depth of 25 cm. Samples were puddled on a 0.5 mm sieve, preserved in a 10% formalin-seawater mixture to which Rose Bengal was added, and sorted by eye. Samples for the analysis of sedimentary parameters were obtained by means of a 0.002 m² corer pushed 5 cm into the substrate. Granulometry was assessed following the methods outlined in Buchanan (1984), with the results expressed as % gravel (>2 mm), % coarse sand (2 mm–710 µm), % medium sand (250 µm–710 µm), % fine sand (63 µm–250 µm), and % silt-clay (<63 µm). Organic matter was assessed by the loss-on-ignition method (Buchanan 1984).

Although not quantified, notes were taken on the presence of any epifaunal species occurring in the vicinity of the samples.

Following enumeration of taxa, the biomass of each taxon was determined using the ash-free dry weight method (Rumohr 1990). On the basis of ranked abundance and biomass figures, Abundance Biomass Comparison (ABC) curves were drawn (Warwick 1986). These curves were statistically compared with the W statistic (Clarke 1990). The following diversity and evenness indices were calculated: S-total number of species, Ind: total number of indi-

viduals, H'-Shannon-Wiener Index, SI-Simpsons' Dominance Index, and J-Pielou's Evenness Index. Formulae of all these indices can be found in Heip et al. (1988).

Univariate community measures such as diversity indices have been shown to be less sensitive to anthropogenic disturbances than multivariate approaches (Keough and Quinn 1991). Hence, a Detrended Correspondence Analysis (DCA) (see Jongman et al. 1987) was performed on the numerical abundance matrix.

RESULTS

The sediment at all three treatments consisted of sand, with only minor portions of either the less than 63 µm and the larger than 2 mm fractions being present. Significantly lower levels of coarse and medium sand and higher levels of fine sand were present at the Lane and Trestle treatments than at the Control treatment (Table 1). In terms of % organic matter, no significant differences existed among the treatments (Table 1).

Of a total of 21 macrobenthic species encountered, only nine showed significant differences in terms of numerical abundance

TABLE 2.
Densities (numbers/m²) and biomass (mg/m²) (mean value ± standard error) of species in treatments, together with results from a Kruskal-Wallis test.

	Density Lane	Trestle	Control	H	Biomass Lane	Trestle	Control	H
<i>Atylus guttatus</i> (Costa)	1 (1)	—	—	2.00 ^{ns}	<1	—	—	—
<i>Bathyporeia guillamsoniana</i> (Bate)	—	1 (1)	20 (5)	18.10**	—	1 (1)	21 (12)	14.31**
<i>Capitella capitata</i> (Fabricius)	18 (7)	47 (16)	—	7.45**	13 (10)	5 (2)	—	6.24**
<i>Cerastoderma edule</i> (L.)	—	2 (2)	2 (2)	1.04 ^{ns}	—	2 (2)	1 (1)	1.04 ^{ns}
<i>Cumopsis goodsiri</i> (van Beneden)	4 (2)	3 (2)	16 (8)	1.42 ^{ns}	21 (13)	2 (1)	5 (2)	1.67 ^{ns}
<i>Eteone longa</i> (Fabricius)	26 (8)	17 (7)	4 (2)	6.14*	42 (20)	34 (29)	3 (1)	6.62*
<i>Eurydice pulchra</i> Leach	—	—	1 (1)	2.00 ^{ns}	—	—	<1	—
<i>Gammarus crinicornis</i> Stock	1 (1)	2 (1)	33 (12)	11.43**	<1	5 (4)	22 (11)	11.47**
<i>Glycera lapidum</i> Quatrefages	—	1 (1)	—	1.04 ^{ns}	—	1 (1)	—	1.04 ^{ns}
<i>Idotea</i> sp.	—	—	2 (1)	4.14 ^{ns}	—	—	2 (1)	4.14 ^{ns}
<i>Lanice conchilega</i> (Pallas)	7 (4)	1 (1)	—	4.11 ^{ns}	61 (50)	23 (23)	—	3.77 ^{ns}
<i>Magelona</i> sp.	2 (1)	—	—	4.14 ^{ns}	20 (20)	—	—	2.00 ^{ns}
<i>Microprotopus maculatus</i> Norman	1 (1)	1 (1)	14 (6)	6.54*	1 (1)	1 (1)	5 (2)	6.54*
<i>Nemertea</i> sp.	3 (2)	1 (1)	—	2.21 ^{ns}	21 (13)	<1	—	3.12 ^{ns}
<i>Nephtys hombergii</i> Savigny	82 (16)	46 (11)	128 (14)	13.01**	116 (25)	88 (23)	222 (30)	11.34**
<i>Owenia fusiformis</i> Della Chiaje	2 (1)	1 (1)	2 (2)	0.46 ^{ns}	11 (10)	1 (1)	10 (10)	0.56 ^{ns}
<i>Scaloplos armiger</i> (Muller)	100 (17)	4 (2)	4 (3)	21.95**	83 (27)	3 (1)	4 (3)	17.55**
<i>Sigalion mathildae</i> Audouin	9 (4)	—	2 (2)	8.03**	71 (30)	—	20 (20)	8.05*
<i>Tellina tenuis</i> da Costa	71 (12)	77 (22)	176 (17)	12.77**	114 (27)	123 (51)	378 (90)	6.80*
<i>Tubificoides benedii</i> (Udekem)	2 (1)	1 (1)	6 (6)	0.46 ^{ns}	1 (1)	1 (1)	1 (1)	0.01 ^{ns}
<i>Venerupis pullastra</i> (Montagu)	—	1 (1)	—	2.00 ^{ns}	—	1 (1)	—	2.00 ^{ns}

df = 2, ** $p < .01$, * $p < .05$, ^{ns} nonsignificant.

TABLE 3.

Mean values (\pm standard error) for biotic indices of treatments, together with results from a pairwise Mann-Whitney test.

	Lane	Trestle	Control	Lane vs. Control	Trestle vs. Control
S	6.70 (0.45)	4.30 (0.52)	5.70 (0.47)	-1.35 ^{ns}	-1.70 ^{ns}
Ind	329.0 (35.9)	198.0 (41.4)	412.0 (32.7)	-1.06 ^{ns}	-2.57*
H'	2.24 (0.10)	1.62 (0.15)	1.92 (0.12)	-2.04*	-1.06 ^{ns}
SI	0.25 (0.02)	0.39 (0.04)	0.32 (0.03)	-2.19*	-0.75 ^{ns}
J	0.84 (0.02)	0.81 (0.04)	0.79 (0.02)	-1.51 ^{ns}	-0.98 ^{ns}

S = total number of species; Ind = total number of individuals; H' = Shannon-Wiener Index; SI = Simpson's Dominance Index; J = Pielou's Evenness Index.

df = 2, * p < .05 or ^{ns} nonsignificant. For explanation of indices see text.

among the three treatments (Table 2). Higher densities of *Nephtys hombergii*, *Bathyporeia guilliamsoniana*, *Gammarus crinicornis*, *Microprotopus maculatus*, and *Tellina tenuis* were encountered in the control treatment compared with the two other treatments. In the Lane treatment, higher densities of *Scoloplos armiger*, *Eteone longa*, and *Sigalion mathildae* were encountered, whereas elevated densities of *Capitella capitata* were observed in the Trestle treatment (Table 2).

The results from the diversity and evenness analysis are ambiguous, although a trend can be observed in the data (Table 3), this is not supported by statistical significance. Both the Shannon-Wiener index (H') and Simpsons' Dominance Index (SI) show a significant difference between the Lane and Control treatments and the total number of individuals (Ind) shows a significant difference between the Trestle and Control treatment. Compared with the values at the Control treatment, the Lane treatment exhibits, on average, a higher number of species which, coupled to a lower number of individuals but more evenly distributed across the species, results in a slightly higher diversity value. In contrast, the Trestle treatment exhibits, on average, a lower number of species which, coupled with a lower number of individuals and a higher dominance, results in a slightly lower diversity value.

Results of the DCA showed a clear distinction among the three

treatments (Fig. 1), with eigenvalues of axes 1 and 2 being 0.40 and 0.20, respectively. All samples from the Control treatment are relatively closely grouped together, characterized by the abundance of the above-mentioned species, which exhibited a higher abundance in these samples. All samples from the Lane treatment are also grouped closely together, characterized mostly by the absence of the species defining the Control treatment group. The Trestle treatment samples exhibit a larger spread than the two other groups, mainly caused by the high variability in density of certain species. High abundances of *C. capitata* influence the positioning of three stations away from the remainder of this group. The Trestle group appears to partly occupy an intermediate position between the Control and Lane groups, with a number of samples being very closely positioned to the Control treatment samples (Fig. 1).

Mean biomass values per species for each of the different treatments (Table 2) showed approximately the same differences among the treatments as the abundance values (Table 2), with all nine species, which showed significant differences in numerical abundance among the treatments, exhibiting a difference in biomass values. A DCA analysis of the biomass matrix approximated the results for the abundance matrix.

Composite ABC curves, based on the mean abundance and

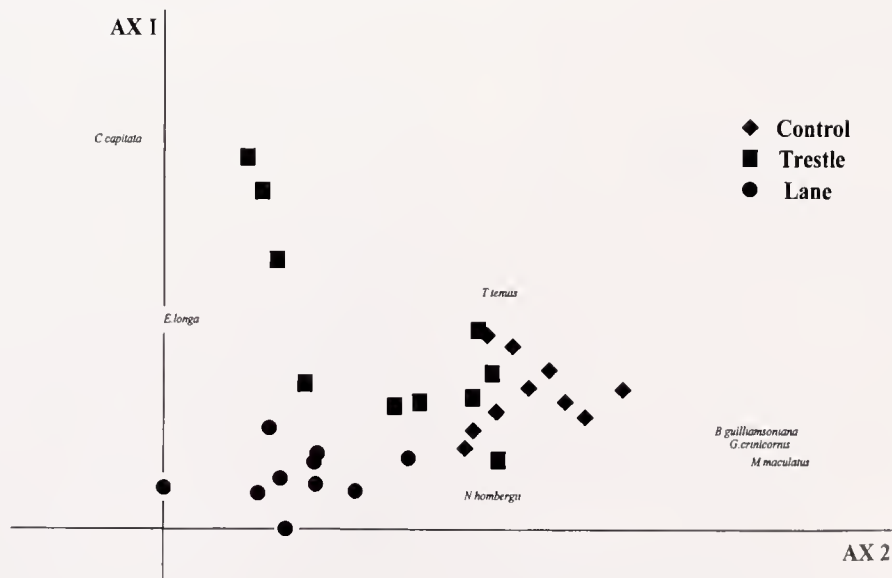


Figure 1. Detrended Correspondence Analysis (DCA) plot of numerical abundance matrix. Only species that show a significant difference in numerical abundance between treatments are shown.

biomass values over all 10 samples in each treatment, are shown in Figure 2. Since in both the Trestle and the Control treatment the biomass curve lies above the abundance curve, it is assumed that these represent a relatively undisturbed community (Warwick 1986). In the Lane treatment the numbers curve lies above the biomass curve, indicative of a more disturbed community compared with the other two treatments. The W statistic values of the Trestle and Control do not differ significantly (Table 4), but a significant difference existed between the W values in the Lane treatment compared with the Control treatment (Table 4). These

results are in agreement with the visual interpretation of the ABC curves.

Relatively high numbers of the epifaunal decapods, *Carcinus maenas* (L.), *Crangon crangon* (L.), and *Palaemon serratus* (Pennant), were noted underneath trestles and in surface depressions in access lanes. In contrast, very few *C. maenas* and *C. crangon* and no *P. serratus* were found at the Control site.

DISCUSSION

In common with other aquaculture practices, intertidal oyster cultivation has the potential to induce organic enrichment. Certainly in view of the high fecal and pseudofecal production (Sornin et al. 1983) and the possibility of altered hydrodynamic forces (Kirby 1994) combined with the large spatial scale of oyster cultivation, this organic enrichment could theoretically be deleterious to oyster growth in addition to causing large scale changes in benthic community functioning. The present results do not, however, indicate that the benthic community at the Trestle treatment is undergoing any form of organic enrichment, as neither elevated levels of organic matter were encountered nor were potential organic enrichment indicator species, such as *C. capitata*, encountered in densities usually associated with organic enrichment (Pearson and Rosenberg 1978). Although densities of *C. capitata* were indeed elevated at both impacted treatment, and especially at the Trestle treatment, this may be more related to the opportunistic nature of *C. capitata* rather than organic enrichment (see Warren 1977). Also, the results from the ABC analysis suggest a relatively undisturbed community (Warwick 1986). In all likelihood the highly dissipative nature of the cultivation site prevents a build-up of organic matter and fines underneath the trestles. This is supported by the fact that the species composition at the noncultivated control area appears to be indicative of a high-energy sandy beach environment (Eleftheriou and Nicholson 1975, Allen and Moore 1978).

On the other hand, the numerical abundance of *C. capitata* was significantly higher at the Trestle treatment, whereas several other species exhibited a lower abundance. In addition, a lower diversity coupled to a higher dominance (although both not statistically significant) was also observed. It thus appears that the presence of oyster trestles induces a minor shift in the total species pool, resulting in some species displacement and changes in numerical abundances of sensitive species. In the absence of any evidence of organic enrichment, the possibility of heightened predation by epifaunal decapods and fishes could be considered as the cause. Certainly, the trestles act as refuges for scavenging crabs and shrimps, with numerous individuals of *C. maenas*, *C. crangon*, and *P. serratus* observed underneath and in between the trestles and these species being virtually absent on the open sands of the adjacent, noncultivated area.

In contrast, all analyses, especially the ordination plot and the ABC analysis, appear to indicate that the Lane treatment is undergoing a slightly more pronounced form of disturbance. Even though the close proximity of the trestles may influence the benthic community structure due to the causal agents of organic deposition, hydrodynamic forces, and predation, in the absence of evidence for organic enrichment and in view of the differences in community structure between both impacted treatments it is postulated that this is evidence of physical disturbance. Frequent heavy vehicle traffic for maintenance and harvesting purposes may indeed alter the surficial sediment matrix due to the combined

Cumulative percentage

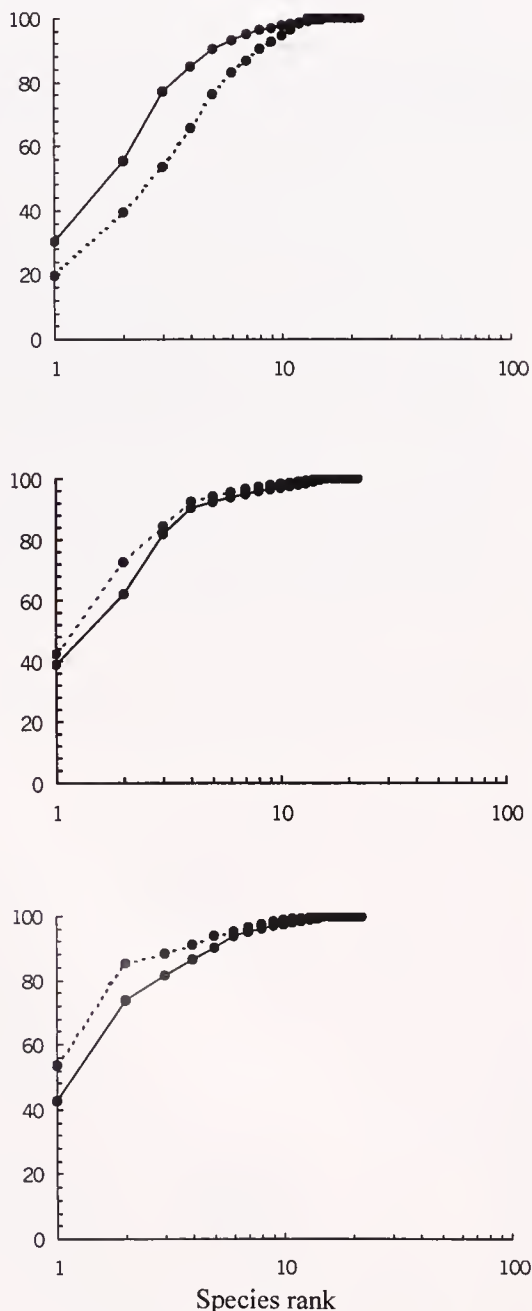


Figure 2. Composite ABC curves for each treatment: Lane (top), Trestle (middle), and Control (bottom). (abundance is shown as a full line, biomass as a dotted line).

TABLE 4.

Mean values of W statistic (\pm standard error) per treatment, together with results from a pairwise t-test.

	Lane	Trestle	Control	Lane vs. Control	Trestle vs. Control
W	0.022 (0.073)	0.101 (0.101)	0.197 (0.050)	-2.30*	-0.85 ^{ns}

df = 18, * p < .05 or ^{ns} nonsignificant.

action of compaction and displacement. This may selectively remove species or reduce numerical abundance of surficial living forms, such as small-bodied crustaceans (*B. guilliamsoniana*, *M. maculatus*, *G. crinicornis*) and shallow, fragile burrowing bivalves (*T. tenuis*). An alternative hypothesis, that this change in community structure is caused by a reduction in hydrodynamic forces, would not appear plausible, as, although a general shift from crustacean dominance to polychaete dominance has been associated with a decrease in hydrodynamic force (Eleftheriou and Nicholson 1975), bivalve molluscs are usually considered to be indicative of more sheltered conditions (Eleftheriou and McIntyre 1976). Hence, the reduced numerical abundance of *T. tenuis* in the Lane treatment as compared with the Control treatment can thus not be solely attributed to a reduction in hydrodynamic action, even though slightly elevated amounts of fine sand were encountered in both the Lane and Trestle treatments. The observed slightly elevated diversity indices at the Lane treatment may indicate an intermediate level of disturbance, both in terms of severity and frequency, which results in a higher species diversity (Connell 1978), akin to the beneficial effects of low organic enrichment (Pearson and Rosenberg 1978).

Previous studies on the potential benthic impact of intertidal oyster cultivation demonstrated clear evidence of organic enrichment

causing reduced macrofaunal abundance and diversity (Castel et al. 1989, Nuges et al. 1996), findings which are not supported by the present study. This is attributed to the more dissipative nature of the present study site, with both the studied sites of Castel et al. (1989) and Nuges et al. (1996) being lower energy sites than the present site, as exemplified by the higher percentages of fines at the nonimpacted sites in those studies.

Generally our findings support the hypothesis that in contrast to the negative environmental effects associated with suspended bivalve cultivation, only minor changes are observed in terms of altered macrofaunal community structure. Nevertheless, a consistent but minor change in macrofaunal community structure was observed in the access corridors, which is assumed to be related to physical disturbance caused by heavy vehicle traffic, resulting in compaction and dispersal of the surficial sediment layers.

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OYSTER RESOURCE ZONES OF THE BARATARIA AND TERREBONNE ESTUARIES OF LOUISIANA

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ABSTRACT A 1:100,000 scale map delineating the subtidal oyster resource zones within the Barataria and Terrebonne estuaries was developed. Strategies to accomplish the task included interviews with Louisiana oystermen and state biologists to develop a draft map, field sampling to document oyster (*Crassostrea virginica*), Dermo (*Perkinsus marinus*), and oyster drill (*Stramonita haemastoma*) abundances, use of historical salinity data to aid in map verification, and public meetings to allow comment on a draft before final map preparation. Four oyster resource zones were delineated on the final map: a *dry* zone where subtidal oysters may be found when salinities increase, a *wet* zone where subtidal oysters may be found when salinities are suppressed, a *wet-dry* zone where subtidal oysters may be consistently found due to favorable salinities, and a *high-salinity* zone where natural oyster populations are predominantly found in intertidal and shallow waters. The dry zone is largely coincident with the brackish-marsh habitat, with some intermediate-type marsh. The wet-dry zone is found at the interface of the brackish and saline marshes, but extends further seaward than up-estuary. The wet zone and the high salinity zones are areas of mostly open water fringed by salt marshes. The dry zone encompasses 91,775 hectares, of which 48,788 hectares are water (53%). The wet zone encompasses 83,525 hectares, of which 66,958 hectares are water (80%). The wet-dry zone encompasses 171,893 hectares, of which 104,733 hectares are water (61%). The high salinity zone encompasses 125,705 hectares, of which 113,369 hectares are water (90%). There is a clear trend of increasing water habitat in the four zones over the past 30 years, and oysters are now cultivated on bottoms that were once marsh. The map should be useful in managing the effects upon oysters of freshwater diversions into the estuaries. It provides a pre-diversion record of the location of oyster resource zones and should prove helpful in the seaward relocation of oysters leases.

KEY WORDS: Oyster, *Crassostrea virginica*, management, Louisiana, wetland loss, coastal restoration, map

INTRODUCTION

Throughout its long history, the Louisiana oyster industry has experienced fluctuating salinities that have influenced the distribution of oysters (Van Sickle et al. 1976, Orlando et al. 1993). In the Barataria and Terrebonne estuaries, many oystermen have adapted to fluctuating salinities by leasing grounds along salinity gradients which allows them to operate in wet, dry, and average years (Melancon et al. 1987, Perret and Chatry 1988, Dugas et al. 1997). The oyster grounds in Barataria and Terrebonne estuaries, 80,000+ hectares, comprise 50–60% of the state's private leases.

As Louisiana develops its strategy of freshwater diversions into the estuaries as one means of stemming wetland losses, displacement and destruction of some oyster-producing grounds is inevitable (Melancon 1990a). We developed a map of the current distribution of the oyster resource zones within the Barataria and Terrebonne estuaries to assist oystermen and state and federal

resource managers in solving problems related to oyster resource displacement.

No oyster resources mapping project of this magnitude has ever been undertaken within the two estuaries. Mackin and Hopkins (1961), using O'Neil's (1949) vegetation map, identified some productive oyster areas within Louisiana. In 1981, the U. S. Dept. of the Interior issued the Ecological Atlas of the Mississippi Deltaic Plain Region delineating some major areas of oyster leases along the Louisiana coast (U.S. Dept. Interior 1981).

MATERIALS AND METHODS

Map Development

The map of oyster distribution was developed by interviewing oystermen and state of Louisiana field biologists. Twenty-seven oystermen with an average experience of 29 years in the industry (range, 8–50 years) were initially interviewed to develop a draft map. Each oysterman was asked to delineate, on a 1:100,000 scale map of the Barataria and Terrebonne estuaries, the zones where natural subtidal oyster populations could always be sustained during wet and dry estuarine conditions (periods of abundant freshwater and low salinities and periods of drought and high inland salinities). Once this zone was drawn, each was asked to draw

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zones where natural subtidal oyster populations could be sustained during wet or dry periods. A high salinity zone was identified (seaward of the wet zone to the Gulf), where cultivated oysters are temporarily bedded and natural populations exist in shallow and intertidal habitats. Oystermen were instructed to draw zones only in those areas where they had knowledge of the oyster resources. A composite draft map, delineating the four oyster resource zones, was then submitted for review to 14 field biologists with the Louisiana Department of Wildlife and Fisheries (LDWF); six biologist recommended slight revisions.

A second draft of the map was submitted to the public at three meetings held in different coastal communities within the estuaries. All 1,200 state-registered oystermen were notified of the meetings by mail and announcements also appeared in eight coastal community newspapers. At each meeting, the 1:100,000 scale second-draft map was reviewed. Any oysterman who wanted to suggest a revision was given a smaller (1:580,000 scale) version of the map upon which to draw his recommended change. Some revisions were a collaborative effort of two or three oystermen. The name, address, years of experience, and the number of acres leased or controlled were recorded for each participant. Forty-five oystermen at the public meetings reviewed the second draft of the map with 14 of them recommending revisions. The suggested revisions were incorporated into a third draft of the map and resubmitted to the field biologists who had reviewed the original draft. No further recommendations were made and the third version of the map became the final draft. A total of 86 oystermen and biologists contributed to the development of the oyster resource map.

The base map used for the drafts and final version was the U. S. Geological Survey's 1:100,000 DLG data file of the Louisiana coast. Boundaries for the map were produced from the Louisiana

Department of Environmental Quality's (LDEQ) water quality segment data file. Boundary lines for each of the four oyster resource zones were digitized and saved using Arc-Info (version 6.1.2) software on a Data General Avion Unix work station located at the Louisiana Department of Natural Resources (LDNR). The final maps were plotted using a Calcomp 58000, 44-inch color electrostatic plotter. Land and water acreage within each oyster resource zone was obtained from the 1988–90 U. S. Fish and Wildlife Service (USFWS) Louisiana habitat data file.

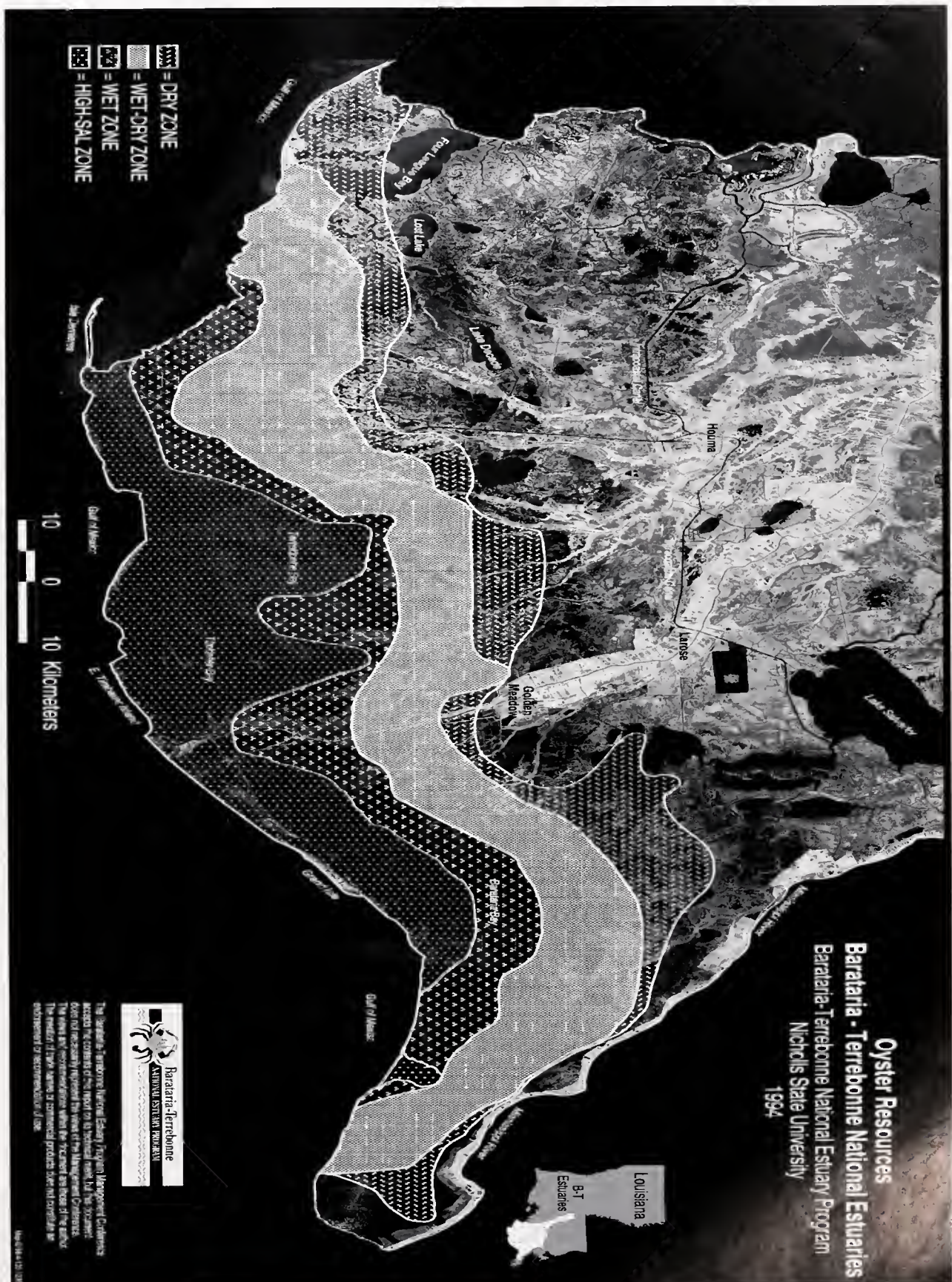
Field Validation

Field validation of the oyster resource zones included a field survey of sites 1–59 (Fig. 1) to document subtidal oyster abundance and size frequency, the presence of the oyster drill, *Stramonita* (= *Thais*) *haemastoma*, and the occurrence of the parasite Dermo, *Perkinsus* (= *Dermocystidium*) *marinus*. Most of the field sites were sampled between May and October 1993, except for station 22 (4/18/93). The parasite survey began on July 24, 1993 and ended on September 23, 1993; thus some of the field sites were revisited. Salinity measurement at each field site was determined with a Hydrolab Surveyor II. A bamboo pole was used to sound the bottom for oysters and shells. Once reef or concentrated shells were discovered, buoys were placed to mark the location. Replicate 1-min tows were taken between buoys with a hand-operated 12-tooth oyster dredge, 61 cm wide. The "length" of live oysters was measured in millimeters (umbo to the most distant point on the lip of the shell) and were assigned to 25 mm size classes.

A subset of 10 live oysters ($N \geq 76$ mm in shell length) were harvested from 24 selected stations (#1, 4, 9, 11, 13, 14, 16, 18, 19, 24, 25, 28, 29, 31, 32, 35, 37, 39, 42, 47, 49, 52, 53, and 56) and



Figure 1. Location of the Barataria and Terrebonne oyster sample stations, #1–#59, and Louisiana Department of Wildlife and Fisheries salinity stations, #60–#81.



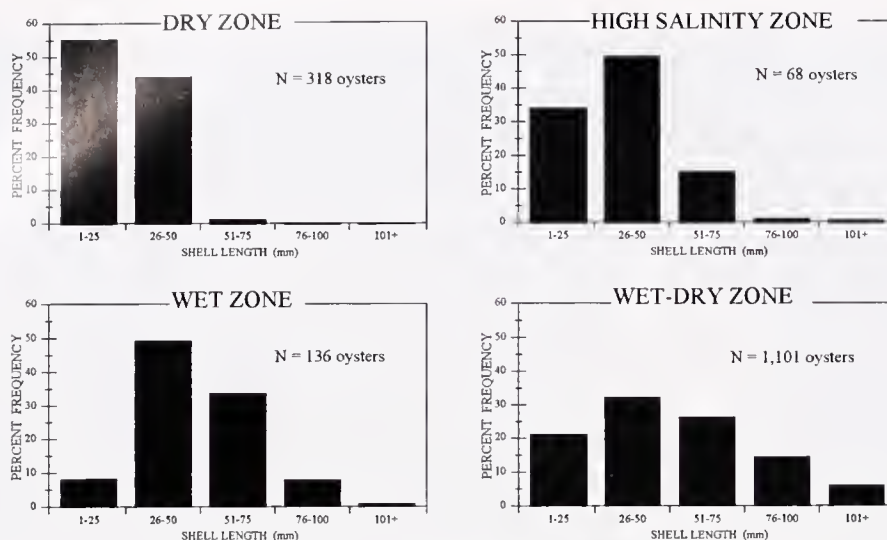


Figure 3. Length-frequency of live oysters in 25-mm size classes from the four resource zones.

analyzed for Dermo (Ray 1966). A parasite intensity code (C) of 0 (uninfected) to 6 (heavily infected) was assigned to each oyster according to the criteria of Quick and Mackin (1971). Percent infection (PI) and the mean population disease intensity or weighted incidence (WI) were calculated, where $WI = (\sum C)/N$ (Mackin 1962).

Besides the 59 field sites, 22 other sites (stations #60–81) were used to validate the delineation of the four oyster resource zones by using records of bottom salinity (Fig. 1). The records were of 5-y (1989–93) monthly mean bottom salinities obtained from the LDWF (Melancon et al. 1994).

RESULTS AND DISCUSSION

Four resource or habitat zones were delineated where, based upon prevailing salinity conditions, subtidal oysters may be found

(Fig. 2). The four zones are a *dry* zone in the upper regions of the estuaries, where subtidal oysters may be found when salinities increase; a *wet* zone in the mid-to lower regions of the estuaries, where subtidal oysters may be found when salinities are suppressed; a *wet-dry* zone in the mid-section of the estuary, where subtidal oysters may be consistently found due to favorable salinities; and a *high-salinity* zone in the lower estuary, where natural oyster populations are predominantly found in intertidal and shallow waters (usually <0.3 m below MLW) and where cultivated populations are often temporarily bedded (Melancon 1990b). When mapped, the zones form an m-shaped pattern, with the zones extending seaward under the influence of freshwater contributions, especially those of the Atchafalaya River, Bayou Lafourche, and the Mississippi River. The more significant flows of the Atchafalaya and Mississippi Rivers extend the wet/dry zone to the edge of

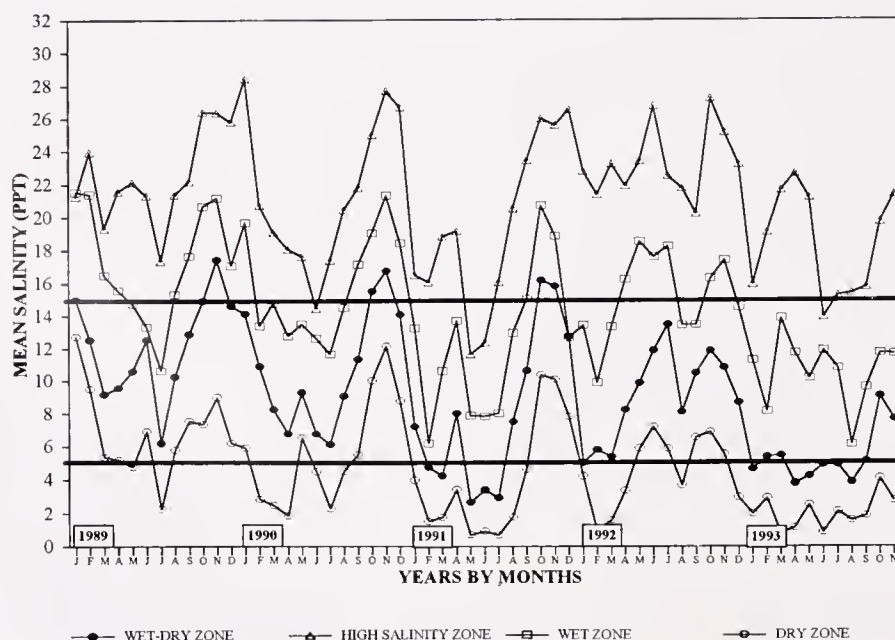


Figure 4. Mean monthly salinities for the four oyster resource zones using Louisiana Department of Wildlife and Fisheries stations #60–81, for the period 1989–93.

TABLE 1.

Weighted incidence (WI) and percent infection (PI) of *Perkinsus marinus*, and mean numbers of drills per dredge tow for the four resource zones.

Oyster Zone	# Stations	Dermo		Oyster Drills #/Dredge Haul
		WI (\pm SD)	PI (%)	
Dry	10	0	0	0
Wet-dry	28	0.1 \pm 0.2	14	0.04
Wet	10	0.1 \pm 0.1	16	0.20
High-salinity	11	0.5 \pm 0.3	65	0.18

the Gulf of Mexico. Conversely, the intrusion of higher salinity waters into the interdistributary bays pushes the zones up-estuary.

Length-frequency plots of live oysters were developed from samples in the four habitat zones (Fig. 3). The wet/dry zone had an even distribution of oyster length classes with about 20% of the oysters having commercial size (≥ 76 mm). The high salinity zone and the wet zone also had an even distribution of length classes, but fewer oysters had reached a commercial size. In contrast, the distribution of oyster length classes in the dry zone was skewed, with no oysters exceeding 76 mm. Most oysters (310 out of 318) collected in the dry zone came from two stations, Raquet Pass (station #1) and Locust Bayou (station #2). Although the number of oysters at the two stations was higher than might be expected at dry-zone sites in a wet year, the sites are adjacent to the open Gulf of Mexico from where recruitment of these oysters likely occurred. In this area, oyster reefs are found offshore, one of the few places that this occurs in the Gulf of Mexico (Price 1954). With the single exception of the Raquet Pass data, these are the results that would be expected if sampling was conducted during a wet cycle, as was

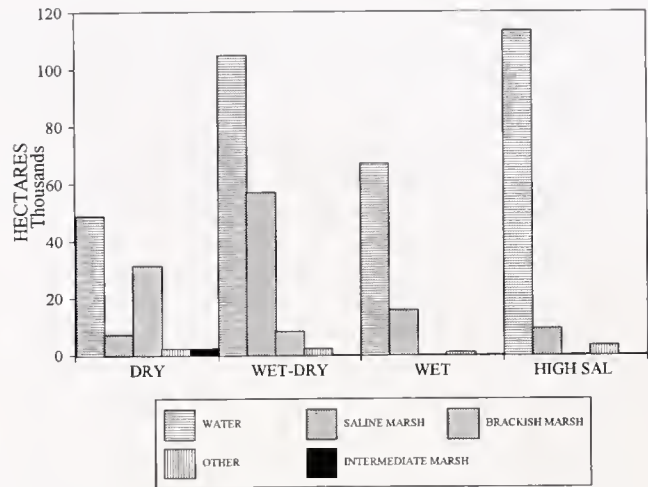


Figure 5. Distribution of habitat types within the four oyster resource zones. U.S. Fish and Wildlife Service habitat data, 1988–90.

done; Figure 4 clearly shows the lower salinities of 1993 and also illustrates the good agreement between the delineated zones and LDWF's bottom salinity measurements from 1989 to 1993. For example, sites within the zone of optimum oyster production (the "wet-dry zone") usually have salinities ranging from 5 to 15 ppt (Ray 1996).

PI of *P. marinus* varied from 0% in the dry zone to 65% in the high salinity zone (Table 1). WI in the dry zone was thus 0.0; it was 0.5 in the high-salinity zone and 0.1 in the intermediate (wet and wet/dry) zones. Oyster drills were absent in the dry zone, in relatively low numbers in the wet-dry zone, and at relatively high numbers in the wet and high-salinity zones (Table 1). The patterns of increasing PI, WI, and drill abundance along the salinity gra-

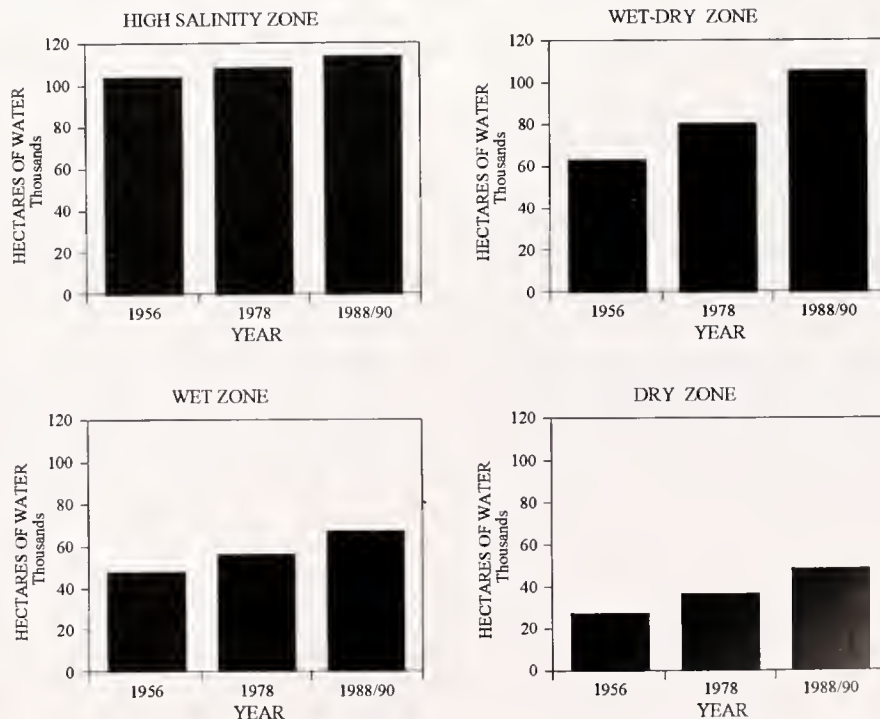


Figure 6. Changes in hectares of water habitat from 1956 to 1988/90 for the four oyster resource zones.

dient and relatively low values for all, are typical of wet year conditions (St. Amant 1938, Gunter 1979, Soniat 1985 and 1996).

The Barataria-Terrebonne oyster resource zones correlate well with coastal vegetation zones, both of which relate to long-term salinity regimes (Fig. 5). The dry zone is largely coincident with the brackish-marsh habitat, with some intermediate-type marsh. The wet-dry zone is found at the interface of the brackish and salt marsh, but extends further seaward than up-estuary. The wet and high-salinity zones are areas of mostly open water fringed by salt marshes. Water habitat, using 1988–90 USFWS Habitat data, is equal to 34% (48,788 hectares) of the dry zone, 61% (104,733 hectares) of the wet-dry zone, 80% (66,958 hectares) of the wet zone, and 90% (113,369 hectares) of the high-salinity zone (Fig. 5).

The USFWS habitat data from 1956, 1978, and 1988/90 show a trend of increasing water habitat in the four zones over the past thirty years (Fig. 6). Since 1958, water acreage has increased 77% in the dry-zone, 67% in the wet-dry zone, 41% in the wet-zone, and 9% in the high-salinity zone. An increase in water acreage is

potentially an increase in available oyster habitat. Oysters now are cultivated on bottoms that were once marsh.

The rationale of the map is that salinity is the main determinant for subtidal oyster presence when all other environmental conditions are met. However, the presence of an oyster population within a resource zone implies neither commercial numbers or quality of oysters. The map is useful in predicting the effects upon oyster habitat of freshwater diversions into the estuaries. It provides a pre-diversion record of the location of oyster resource zones and should prove helpful in the seaward relocation of oysters leases. The state of Louisiana and the oyster industry are presently using the map for this purpose.

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UNDERSTANDING THE SUCCESS AND FAILURE OF OYSTER POPULATIONS: THE IMPORTANCE OF SAMPLED VARIABLES AND SAMPLE TIMING

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ABSTRACT One of the primary obstacles to understanding why some oyster populations are successful and others are not is the complex interaction of environmental variables with oyster physiology and with such population variables as the rates of recruitment and juvenile mortality. A numerical model is useful in investigating how population structure originates out of this complexity. We have monitored a suite of environmental conditions over an environmental gradient to document the importance of short time-scale variations in such variables as food supply, turbidity, and salinity. Then, using a coupled oyster disease population dynamics model, we examine the need for short time-scale monitoring. We evaluate the usefulness of several measures of food supply by comparing field observations and model simulations. Finally, we evaluate the ability of a model to reproduce field observations that derive from a complex interplay of environmental variables and address the problem of the time-history of populations. Our results stress the need to evaluate the complex interactions of environmental variables with a numerical model and, conversely, the need to evaluate the success of modeling against field observations of the results of complex processes. Model simulations of oyster populations only approached field observations when the environmental variables were measured weekly, rather than monthly. Oyster food supply was estimated from measures of total particulate organic matter, phytoplankton biomass estimated from chlorophyll *a*, and total labile organic matter estimated from a regression between chlorophyll *a* and total labile carbohydrate, lipid, and protein. Only the third measure provided simulations comparable to field observations. Model simulations also only approached field observations when a multiyear time series was used. The simulations show that the most recent year exerts the strongest influence on oyster population attributes, but that the longer time-history modulates the effect. The results emphasize that year-to-year changes in environment contribute substantially to observed population attributes and that multiyear environmental time series are important in describing the time-history of relatively long-lived species.

KEY WORDS: *Crassostrea virginica*, modeling, *Perkinsus marinus*, population dynamics, seston

INTRODUCTION

The American oyster, *Crassostrea virginica*, is notable for its ability to maintain commercial populations over a wide latitudinal range and over a wide range of local environmental gradients, chief of which are gradients in salinity, turbidity, and food supply. Although the American oyster is a well-studied species, the interaction of salinity, temperature, and food supply on commercially productive populations is still poorly understood and the degree to which other variables, such as current flow, are important under certain conditions remains largely unknown. Nevertheless, the interaction of salinity, turbidity, and food supply with the annual temperature cycle and their joint influence on the susceptibility of the American oyster to disease may explain much of the yearly variability observed in oyster populations.

The interaction of these variables, however, produces complex and sometimes counterintuitive results. Some high-salinity populations are productive, despite high disease intensities and heavy adult mortality, conditions that produce local extinctions elsewhere. Some low-salinity populations exhibit poor growth and low-productivity, despite the absence of disease, conditions that produce commercially important populations elsewhere. Modeling of oyster population dynamics has indicated that variations in food

supply and turbidity, independent of salinity, may explain these diverse observations (Hofmann et al. 1992, Powell et al. 1994b). Food supply and turbidity are contrapuntal variables in their influence on oyster ingestion. Increased food supply normally results in increased ingestion, until ingestion is restricted by gut passage time (Hughes 1980). Increased turbidity reduces ingestion rate by reducing food quality and reducing filtration rate (Powell et al. 1992b, Loosanoff and Tommers 1948). Thus, food supply and turbidity are important mediators of population success over a wide range of environmental conditions.

The persistence and productivity of oyster populations is determined by a complex interaction of such environmental variables as temperature and food supply with oyster physiology and with such population variables as rates of recruitment and juvenile mortality. Modeling studies have emphasized this complexity (Powell et al. 1992b, Powell 1994b, Buxton et al. 1981, Soniat and Brody 1988). The complexity is also apparent in experimental studies evaluating the importance of many variables in population success (e.g., Buxton et al. 1981, Bayne et al. 1988, Ólafsson et al. 1994).

Combining field observation and numerical modeling to investigate the complex basis for population structure rests first on the collection of data necessary to describe the environmental and

biological milieu. Typically, data collection faces sampling limitations imposed by time and resources. Four aspects of this problem are pertinent. First, in many cases, only a limited suite of environmental and biological variables can be measured; however, the choice of which variables to measure may not be easily determined. Some may be more important in some places than in others, and the simplest to measure may not be the most important. Second, in some cases, as in the case of food supply, uncertainty exists in what should be measured. Various estimators of food supply, for example, include total organic carbon, total organic nitrogen, chlorophyll *a*, and lipid/carbohydrate/protein (Soniata et al. 1984, Soniat and Ray 1985, Wilson-Ormond et al. 1997). The assimilation efficiency of oysters varies profoundly across a variety of food resources differentially targeted by these measures (Langdon and Newell 1990, Powell et al. 1992b, Tshikhon-Lukanina 1982). Third, the frequency of sampling is an issue. Studies normally target sampling on once per month intervals. Fegley et al. (1992) and Wilson-Ormond et al. (1997) have shown the importance of short time-scale changes in environmental variables for oyster populations. Hofmann et al. (1992) emphasized the importance of the timing of phytoplankton blooms relative to the seasonal temperature cycle. Thus, events on less than month time-scales may be crucial in understanding population success. Fourth, on the other hand, Ulanowicz et al. (1980) and Powell et al. (1996), among others, discussed the importance of the time-history of the population in shaping population structure. Thus, the length of the time series may be crucial in interpreting differences in population structure. Any population represents the time-history of environmental events over at least the last few generations. The degree to which any one year's events control future population structure is not well understood, but certainly disease epizootics seem to have a multiyear history of development and decay (Powell et al. 1996). This generational effect will restrict the information gained from comparisons of simultaneously run biological and environmental sampling programs, while lengthening the necessary sampling period to encompass a longer time series.

The purpose of our investigation is threefold. First, we monitored a suite of environmental conditions over an environmental gradient to document the importance of short time-scale variations in such variables as food supply, turbidity, and salinity. Then, using a model, we examined the need for short time scale monitoring. Second, we evaluated the usefulness of several measures of food supply by comparing field observations and model simulations obtained using them. Finally, we evaluated the ability of a model to reproduce field observations that derive from a complex interplay of environmental variables, and we sought to determine the adequacy of our choice of monitoring variables. Along the way, we addressed the problem of generational memory or the time history of populations. Our results stress the need to evaluate the complex interactions of environmental variables with a numerical model and, conversely, the need to evaluate the success of modeling against field observations of the results of complex processes.

MATERIALS AND METHODS

Sample Sites and Protocol

Environmental variables, seston composition, and oyster population parameters were sampled from April 1992 to March 1993 at three sites in the Terrebonne Basin of southcentral Louisiana. Sample reefs along a salinity gradient were chosen to provide

accessible, unharvested, unplanted, subtidal, and persistent populations of oysters. All sites were adjacent to Bayou Petit Caillou, a local source of fresh water. Site 1 (Savin Canal) is a low-salinity, protected (bayou-like) habitat, water depth 0.3 to 0.6 m. Site 2 (Bay Cocodrie) is an exposed bay-edge, low-salinity location with a depth of 0.6 to 0.9 m. Site 3 (Bay Tambour) is higher in salinity and semiprotected (bayou side of a marsh island in an open bay), water depth 0.3 to 0.6 m.

Environmental Variables

Environmental variables were measured weekly. Water temperature was measured to the nearest 0.1°C with a mercury thermometer. Salinity was determined (nearest 0.5‰) using a refractometer (Behrens 1965).

Seston

Water was sampled weekly from 0.3 m above the reef with a hand-operated peristaltic pump. Between 100 and 250 mL (depending upon turbidity) was filtered through 47-mm Gelman A/E glass fiber filters. The filters and filtrate were dried for 1 h at 103°C to obtain seston dry weight and subsequently ashed at 550°C for 15 min to determine particulate inorganic and particulate organic matter (POM) (American Public Health Assoc. 1971). Twenty-five mL of water was filtered through 25-mm Whatman GF/F glass fiber filters, extracted in 60:40 v:v 90% acetone: dimethyl sulfoxide, and read on a Turner Designs Model 10 fluorometer to measure chlorophyll *a* (Shoef and Lium 1976).

Food potentially available for oysters was estimated in three ways. First, measured POM was used directly. Second, chlorophyll *a* was converted to phytoplankton biomass using a chlorophyll-to-mg carbon conversion of 40 (Parsons et al. 1961) and a mg carbon-to-mg dry weight conversion of 2.14 (Widdows et al. 1979) as described by Wilson-Ormond et al. (1997). Third, food was estimated using a regression equation relating total labile carbohydrate, total protein, and total lipid to chlorophyll *a* obtained from studies in Galveston Bay, Texas (Soniata et al. 1984).

$$\text{Food} = \text{Total carbohydrate} + \text{Total lipid} + \text{Total protein}$$

$$= 0.088 * \text{chlorophyll } a + 0.520$$

where chlorophyll *a* is in $\mu\text{L L}^{-1}$, and food is in mg DW L^{-1} . This third method has the interesting attribute of increasing oyster food supply above that estimated by chlorophyll *a* and also increasing food supply disproportionately during the winter, when chlorophyll *a* values are normally low.

Oysters

About 0.13 m³ of reefal material (enough to fill a 13.2-L bucket) was collected monthly by tonging. Live oysters, boxes (dead articulated shells), and single shells were separated. Live oysters were tallied and assigned to a 25 mm size class (e.g., 0 to 24 mm, 25 to 49 mm). Shells and live oysters were examined for the presence of spat.

Ten commercial-size (≥ 76 mm) live oysters were culled, cleaned of attached epifauna, and anterior-to-posterior length was measured to the nearest mm. Displacement volume before and after shucking was used to calculate mantle cavity volume (Galtsoff 1964). Gonadal thickness and adductor muscle diameter were measured with vernier calipers (nearest 0.1 mm) to calculate gonadal index.

$$\text{Gonadal Index} = \frac{\text{average gonadal thickness (mm)}}{\text{average diameter adductor muscle (mm)}} \cdot 100$$

(Soniati and Ray 1984). Sex was determined by blotting gonadal material onto a glass slide and observing the tissue at 100 X. Dry weight was determined by drying at 80 to 85°C to constant weight. Condition index was calculated as follows.

$$\text{Condition Index} = \frac{\text{oyster dry weight (g)}}{\text{mantle cavity volume (mL)}} \cdot 100$$

(Hopkins 1949).

A small piece of mantle tissue (about 4 mm²) was used to assay for *Perkinsus marinus* (Ray 1966). Infection intensity was scored using Mackin's (1962) 0-to-5-point scale as modified by Craig et al. (1989). Population infection intensity was calculated as weighted incidence (WI).

$$\text{WI} = \frac{\sum \text{Mackin's disease code number}}{n}$$

Oyster *Perkinsus marinus* Model

The oyster population dynamics model consists of separate components for the postsettlement oyster population and *Perkinsus marinus*. The two model components are coupled by the relationships that describe the removal of oyster energy by the parasite to support its metabolic needs, relationships that relate rates of parasite cell division and mortality to host mortality, and the influence of *P. marinus* on oyster physiology. The postsettlement component, as described by Powell et al. (1995), consists of a size-structured model that includes the processes regulating growth, reproduction, and death of oysters from newly settled juveniles to adults. These processes include assimilated ingestion as it depends on filtration rate, ambient food supply, and assimilation efficiency; filtration rate as a function of oyster size, temperature, salinity, turbidity, and current flow; respiration as it depends upon size, temperature, and salinity; and the apportionment of net production into somatic and reproductive growth as a function of temperature and time of year. The *Perkinsus marinus* component, as described by Hofmann et al. (1995), consists of processes controlling cell division and cell mortality as a function of temperature, salinity, and cell density; transmission rate as a function of oyster population density, *P. marinus* prevalence, and *P. marinus* infection intensity; and host mortality as a function of cell density. A flowchart of the coupled oyster disease model appears as Figure 1.

The model was solved numerically using an implicit (Crank-Nicolson) tridiagonal solution technique with a 1-day time step. Environmental forcing factors in the model are salinity, temperature, current flow, food supply, and turbidity. Environmental variables were input into the model from measured time series at each of the three sites. Daily values used by the model were obtained by linear interpolation between each measurement and the next. The model simulates oyster biomass, rather than length. Model results were expressed as length using a dry weight-to-length relationship obtained from the three studied populations.

$$\text{Dry weight(g)} = 2.4 \times 10^{-6} \text{ Length(mm)}^{2.935}$$

All simulations began on January 1 (Julian day 1) or May 20 (Julian day 140) and ran for 6 years. Each simulation was initialized with an oyster size-frequency distribution representative of the sampled site or by permitting the settlement of spat on day 140. The oyster population was initialized with a *P. marinus* prevalence

of 50% to reduce the time required to bring the model into equilibrium with the environmental variables. Typically, this took about 12 to 18 months of simulation. Thus, discussion focuses on the final 4 years of the 6-year simulation. Specific variables set for each simulation are shown in Table 1.

RESULTS

Field Measurements

Environmental Variables

Temperature was similar at all three sites and varied from 12°C in December to 31°C in July (Fig. 2). Salinities were highest at Bay Tambour (site 3, Fig. 2c), often $\geq 5\text{‰}$ above sites 1 and 2. Salinity remained above 10‰, except during the winter months. Salinities were similar at sites 1 and 2 and hovered around 10‰ except during the winter, when salinities below 5‰ were frequently recorded (Fig. 2a,b). Salinity was more variable on short time scales in Bay Cocodrie (site 2).

Site 1 was typically the least turbid (Fig. 3a). Total seston rarely exceeded 60 mg L⁻¹. No seasonal pattern was evident. Total seston was generally higher at sites 2 and 3, often exceeding 40 mg L⁻¹ (Fig. 3b,c). Again, no seasonal pattern was evident.

Food Supply

A moderately distinct bloom occurred during May to July at all three sites. Chlorophyll a concentrations exceeded 20 µg L⁻¹ during some of this period (Fig. 3), however highest values did not occur simultaneously. Values were higher earlier (in May) in Bay Tambour (site 3), but rose to peak values in July at the lower-salinity sites (Savin Canal, Bay Cocodrie). Concentrations fell to persistently low levels (<10 µg L⁻¹) for the remainder of the year at all three sites. No fall bloom was evident at any site.

Particulate organic matter was correlated with total seston, rather than chlorophyll (POM vs. seston: $r^2 = 0.86, 0.94, 0.89$; POM vs. chlorophyll: $r^2 = 0.02, 0.10, 0.05$; stations 1, 2, and 3, respectively [Fig. 4]). Values routinely were 10% or less of total seston.

Oysters

Most oysters were 25 to 99 mm long (Fig. 5) and the size-frequency distribution remained stable throughout the year. No oysters exceeded 150 mm. Site 1 had the smallest oysters, on average. By the end of the study, oysters were somewhat larger at site 3 than at sites 2 or 1. Juvenile oysters (0–24 mm) were most abundant in early spring and late summer, and were normally most abundant at site 3.

At all stations, gonadal index peaked in April or May and was lowest in November (Fig. 6). Second, smaller peaks occurred in July at site 1, August/September at site 2, and September at site 3. Sex was indeterminant from November through February coincident with low values of gonadal index; otherwise, the commercial-size oysters were predominantly female. Condition index paralleled gonadal index in being highest in April/May and lowest in November (Fig. 6). However, condition index was relatively lower during the second peak in gonadal index than during the spring, and particularly low at site 3.

Perkinsus marinus prevalence was highest at the higher-salinity site (Bay Tambour, site 3) and lowest at site 2 (Bay Cocodrie), where frequent low-salinity events occurred. Prevalence rarely

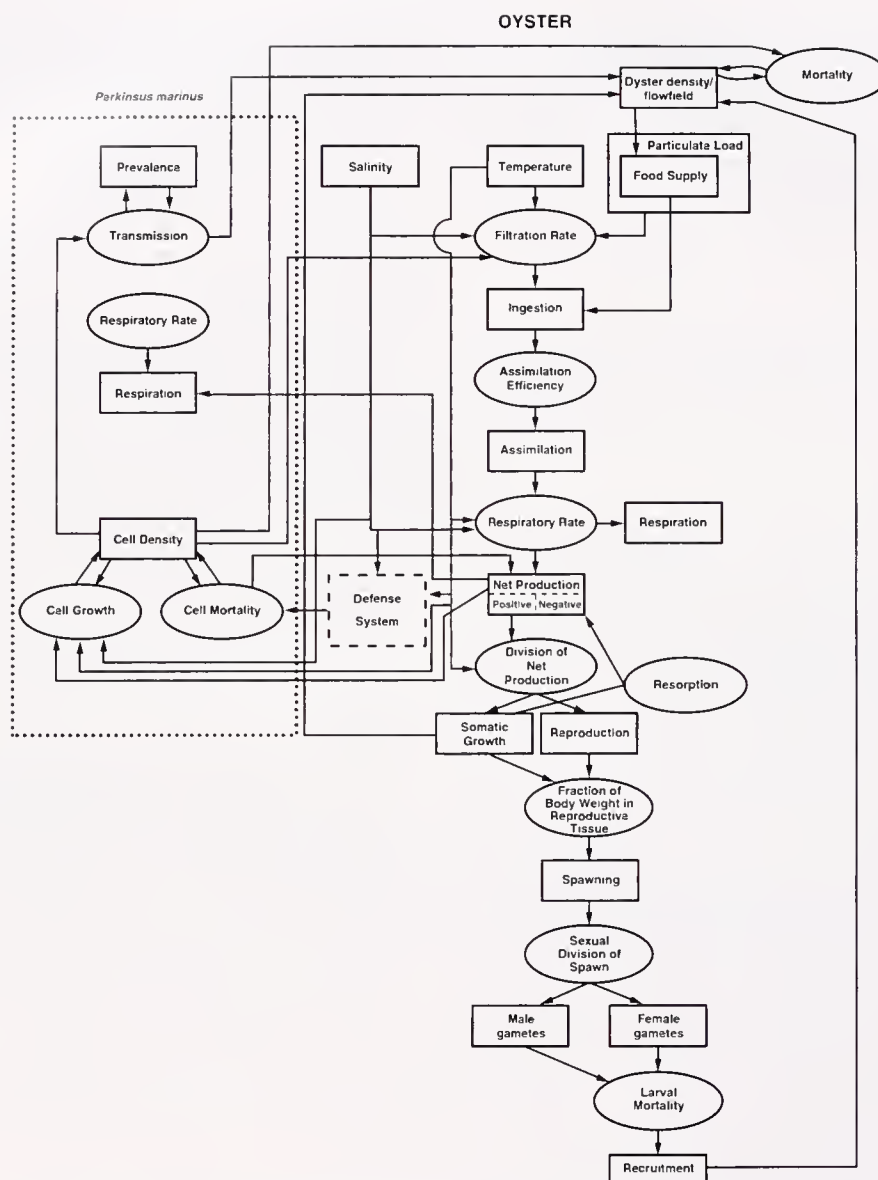


Figure 1. Flow chart for the *Crassostrea virginica*-*Perkinsus marinus* model described by Powell et al. (1995) and Hofmann et al. (1995). Note the following corrections to Table 3 of Hofmann et al. (1995): $\beta = 3.2454 \times 10^8$, $\epsilon = 9.57 \times 10^{-10}$, $\zeta = 1.16 \times 10^{-4}$.

dropped below 50% at site 3 and never rose above 40% at site 2 (Fig. 7). Weighted incidence followed the same general trend, never exceeding 0.35 at site 2 and 0.9 at site 1, but averaging above 0.5 at site 3. Weighted incidence was lowest during the winter at sites 1 and 3. Weighted incidence was much more variable at site 2, coincident with the more variable salinity at this site. Overall, *P. marinus* prevalence and infection intensity followed the expected pattern with temperature and salinity at these sites. Lowest infection levels occurred at the lower salinity sites and during the colder periods of the year.

Model Simulations

Food Supply

The first set of simulations examined the adequacy of various measures of food supply. A crucial prediction, in judging the adequacy of an input variable describing food supply, is the size

frequency of the simulated adult population. In oysters, adult size is environmentally controlled, and, within a local area, food supply will be a significant determining factor of adult size (Hofmann et al. 1994). A second important parameter is the spawning season and the pattern of spawns. The amount of energy devoted to gamete production is sensitive to oyster net production, which is largely determined by the availability of food (Hofmann et al. 1992).

The size frequencies of three simulated populations obtained using the environmental conditions for site 3 (Bay Tambour) and the three different food supplies are shown in Figure 8. Other model parameters were set, as given in Table 1. Simulations for sites 1 and 2 yielded qualitatively similar results. In comparison to the actual population shown in Figure 5, particulate organic matter yields a simulated size frequency of adults much too large; standard estimates of phytoplankton biomass from chlorophyll yields a simulated size frequency much too small. Using an estimate of

TABLE 1.
Parameters used for simulations.

Simulation	Site ^a	Day 1 ^b	Size Frequency ^c	Food ^d	<i>P. marinus</i>	Rate of Recruit ^e	Mortality Size Range ^f	Rate of Mortality ^g	Sampling Frequency ^h	Figure
1.	1	b	a	POM	Absent	0	none	0.	week	8,9
2.	2	b	a	POM	Absent	0	none	0.	week	8,9
3.	3	b	a	POM	Absent	0	none	0.	week	8,9
4.	1	b	a	Chl	Absent	0	none	0.	week	8,9
5.	2	b	a	Chl	Absent	0	none	0.	week	8,9
6.	3	b	a	Chl	Absent	0	one	0.	week	8,9
7.	1	b	a	LPC	Absent	0	none	0.	week	8,9,10
8.	2	b	a	LPC	Absent	0	none	0.	week	8,9,10
9.	3	b	a	LPC	Absent	0	none	0.	week	8,9,10
10.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	week	11,12,13
11.	3	a	b	LPC	Present	2.	1-4	.0208	week	14,15,16
12.	1	a	b	LPC	Present	2.	1-4	.0208	week	14,17
13.	1	a	b	LPC	Present	6.	1-4	.0192	week	15,16
14.	1	a	b	LPC	Present	2.	1-3	.0128	week	17
15.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	mon	18,19,20
16.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	week	18,19,20
17.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	1st	21,22,23,24
18.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	2nd	21,22,23,24
19.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	3rd	21,22,23,24
20.	3,2,1	a	b	LPC	Present	3.9	1-4	.0192	4th	21,22,23,24

^a Multiple sites indicate that the site environmental time series were used sequentially in the order listed, for one year at a time, during the model run.

^b a, simulation begun on January 1; b, simulation began May 20.

^c a, simulation initialized with a settlement of spat on day 140; b, simulation initialized with a population size-frequency distribution typical of the simulated site.

^d POM, particulate organic matter; Chl, phytoplankton biomass directly estimated from chlorophyll; LPC, food estimated from a regression between chlorophyll and total lipid, labile carbohydrate, and protein.

^e Fraction recruited listed as the number of successful recruits per 10⁶ larvae spawned.

^f Size classes exposed to postsettlement mortality. Size class designation refers to the 11 size classes as defined in Figure 8, none, no postsettlement mortality.

^g in day⁻¹.

^h Mon, one sample per month (average of the four weekly samplings) used to define conditions present on the 15th day of each month and daily values obtained by linear interpolation between these monthly values; week, the weekly measurements with conditions imposed on the day of collection with linear interpolation between collection times; 1st, 2nd, 3rd, 4th, the weekly value for that week assumed to be a monthly sample defining conditions present on the 15th day of each month and daily values obtained by linear interpolation between these monthly values.

food that includes a nonchlorophyll-explained food resource based on lipid, labile carbohydrate, and protein yields a size frequency that is similar to field observations.

We compare the pattern of spawning and the amount of gonadal tissue present in simulated oyster populations at site 3 (Bay Tambour) under the three different food supplies (Fig. 9). Simulations for sites 1 and 2 yielded qualitatively similar results. The actual population shown in Figure 6 spawned in May to July and possibly in September to October, as suggested by the gonadal index (Fig. 6). Condition index was highest in April to June. The spawning season for a simulated population in which particulate organic matter was used for food begins and ends too late in the year (Fig. 9). Spawning season in the simulated populations using chlorophyll directly to estimate phytoplankton biomass or as a surrogate for total lipid, labile carbohydrate, and protein better approximates field conditions. Gametic tissue, however, is no longer present in the simulated population at the end of August, when the direct conversion of chlorophyll to phytoplankton biomass is used to estimate the primary food resource. Using total labile carbohydrate, protein, and lipid extends the time gametic tissue is present into October, as seen in the field, and suggests that the September/October decline in gonadal index (Fig. 6) may be

attributable to gonadal resorption rather than spawning. Once again, the simulation in which food supply contains a nonchlorophyll-explained portion based on measures of total lipid, labile carbohydrate, and protein, provides results most similar to field observations.

Comparison Across Environmental Gradients

For these simulations, we assumed that the only difference between the three sampling sites was in the time-history of the environmental variables that were measured: temperature, salinity, food supply, and turbidity. All other processes, such as larval survivorship and juvenile mortality, were kept constant (Table 1). The three sites represent a substantial salinity gradient (from 2 to 1 to 3, Fig. 2). Turbidity is highest at site 2 (Fig. 3). Lower salinity and high turbidity should restrict adult size at site 2. By the end of the study, oysters were somewhat larger at site 3 than at sites 2 or 1, as might be anticipated from the salinity gradient (Fig. 5). Increased turbidity at site 2 did not, however, seem to influence adult size. The simulated oyster population size-frequency distributions conform to both of these observations (Fig. 10).

Comparing simulated reproductive effort with observation is

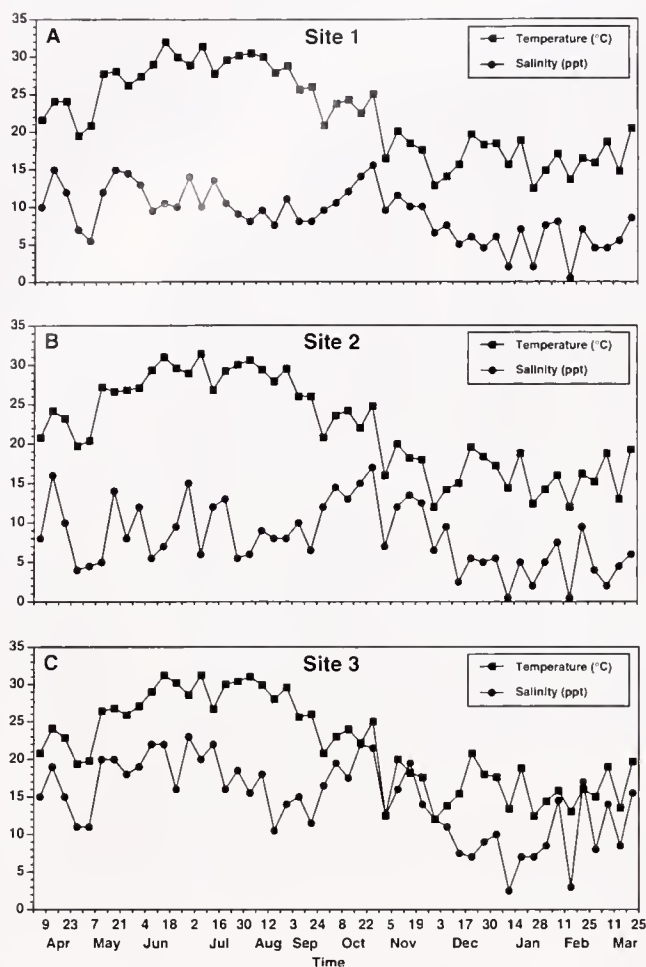


Figure 2. Field measurements of salinity and temperature; (A) site 1, Savin Canal; (B) site 2, Bay Cocodrie; (C) site 3, Bay Tambour.

difficult, because the number of gametes spawned is readily calculated in the model, but not readily measured in the field. Field observations of gonadal index must be used to evaluate spawning. Simulating the reproductive cycle is also more complex than simulating adult oyster size, because spawning and fecundity are sensitive to a large number of environmental and biological factors. *P. marinus* infection intensity, for example, can have a significant impact on fecundity. Therefore, the influence of the environmental gradient on an oyster population infected with *P. marinus* was examined by sequentially exposing the population to environmental conditions for the three sites 1 year at a time. The basis for this sequential juxtaposition of time series is discussed in a subsequent section. Spawning season is longer and fecundity is higher in the simulated population during the years when the site 3 time series was used (Fig. 11). Gonadal index averages lower at site 2. Spawning season inferred from gonadal index seems to be shortest at site 1. The early fall increase in gonadal index at sites 2 and 3 is observed in simulations of sites 2 and 3. Thus, to the extent that a comparison of gonadal index to fecundity permits, the simulated population follows trends observed in the field data.

P. marinus prevalence and infection intensity usually are strongly influenced by salinity gradients. In particular, both should be lower at low salinities. The simulated *P. marinus* prevalences (Fig. 12) and infection intensities (Fig. 13) show these general trends. Simulated *P. marinus* summer prevalence averages be-

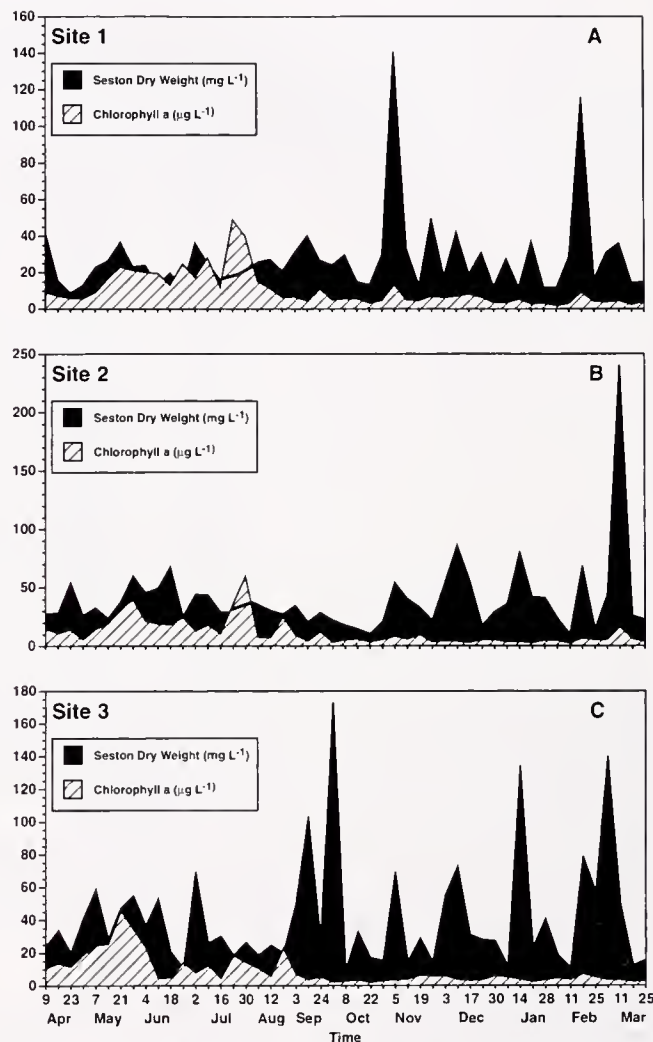


Figure 3. Field measurements of chlorophyll a and total seston; note that the y-axis scale differs between plots; (A) site 1, Savin Canal; (B) site 2, Bay Cocodrie; (C) site 3, Bay Tambour.

tween 60 and 80% at site 3, about 20% at site 2, and between 30 and 50% at site 1, very similar to field observations (Fig. 7). Summer infection intensity averages 2 to 3 at site 3, less than 0.5 at site 2, and about 1 at site 1 for market-size oysters; in each case slightly above field observations. Infection intensity in the simulated entire population is even more similar to field observation, particularly for sites 2 and 3 (Fig. 7).

Overall, the model reasonably approximated adult size, spawning pattern, and population disease intensity across the salinity gradient represented by sites 2, 1, and 3.

Biological Processes and Population Instability

Qualitatively, the environmental gradients are adequately simulated by the model. However, achieving the degree of similarity between simulation and observation shown in Figures 10 to 13 required cycling the oyster population sequentially through the environmental time series from the three sites (Table 1). For simplicity, we present results for only one of a number of possible sequences (i.e., 3, 2, 1, Table 1). Any population is a product of the time-history of environmental change. Oyster populations are in-

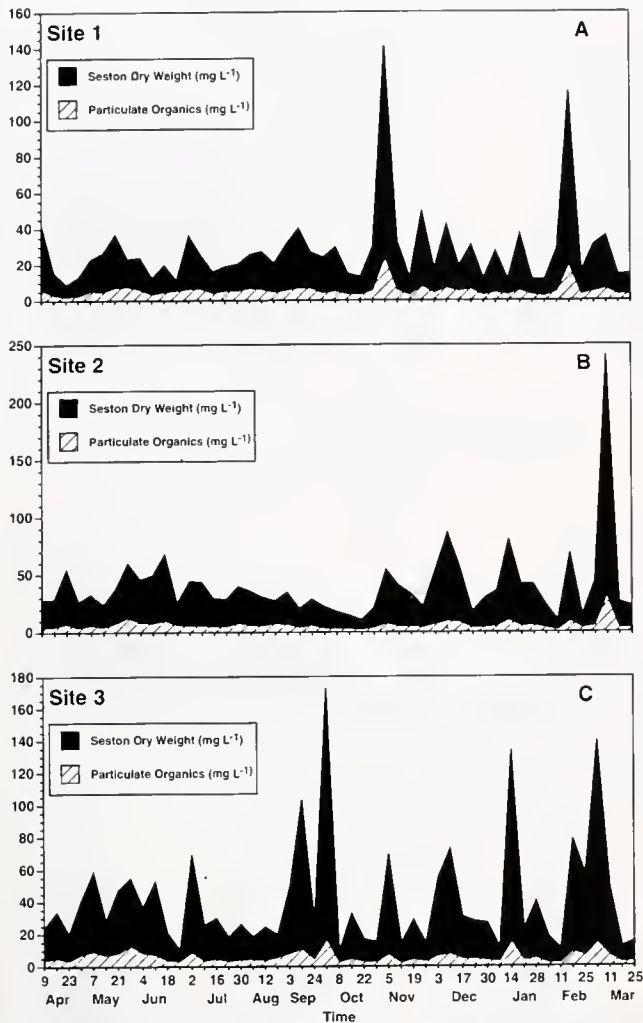


Figure 4. Field measurements of particulate organic matter and total seston. Note that the y-axis scale differs between plots: (A) site 1, Savin Canal; (B) site 2, Bay Cocodrie; (C) site 3, Bay Tambour.

herently unstable, either expanding or contracting based on variations in environment and external forces, such as larval influx from distant brood stock. Realistic simulations, such as depicted in Figures 10 to 13, require continual transitions between conditions promoting expansion and contraction.

Figure 14 shows an example of simulated populations exposed continuously to the same set of conditions over several years. Local biological factors did not vary among these simulations; e.g., the same rate of larval survival and juvenile mortality. Environmental conditions at site 1 result in population extinction over several years; whereas, environmental conditions at site 3 result in population expansion. Population stability can be achieved by varying such biological factors as the rates of recruitment and mortality. If, for example, the rate of subadult recruitment at site 1 is higher than site 3 because of a proportional increase in recruitment and lower juvenile mortality, site 1's population persists over several years, just as does site 3's (Fig. 15). Thus, modifying the rate of subadult recruitment achieves much the same result as was achieved by cycling the environmental time series. However, Figure 16 shows that both simulations result in a slow increase in *P. marinus* infection intensity, unlike field observations. By year 6,

epizootic conditions are evident in both simulated populations. Variations in the rate of juvenile mortality as a result of varying predator abundance can also result in oyster populations that expand or contract over a period of years (Fig. 17). Varying predator abundance can also achieve stability in population abundance and size structure (simulation not shown), but, once again, *P. marinus* infection intensity does not stabilize.

Overall, these three sets of simulations show that several consecutive years of the same environmental conditions always result in a simulated oyster population that fails to resemble field observations of all population variables. Varying such biological factors as the rates of recruitment and mortality markedly improves the simulation, but does not result in an acceptable simulation of disease. Only if the environmental time series include substantial year-to-year environmental change, do the simulated populations correspond closely to field observations at the three Terrebonne Basin sites.

Time Scale of Monitoring (Weekly vs. Monthly)

Most routine sampling is conducted monthly. For many studies, weekly sampling would be feasible. Technological advancement has permitted the sampling of some variables much more frequently. The question is, then, how frequently are samples needed to resolve the processes controlling oyster population structure adequately? The effect of measurement frequency was tested by inputting weekly or monthly (calculated as the mean of the 4 weekly measurements) observations and interpolating these to daily values (Figs. 18–20). In all cases, for all important biological attributes used to characterize an oyster population (e.g., oyster density, spawning pattern, *P. marinus* infection intensity), monthly averages produce simulated populations that compare poorly to field observations. The monthly values tend to overestimate population abundance (Fig. 18), reproduction (Fig. 19), and disease intensity (Fig. 20). Weekly measurements produce simulated populations much more similar to field observations (Figs. 18–20).

The Importance of Sampling Time

In many routine monthly monitoring regimens, the time of sampling is not firmly set. Nevertheless, the single measurements taken are usually assumed to be indicative of conditions during the entire month. We examined the consequences of ignoring the time of month that the measurement was made in a series of simulations in which the observations from each of the 4 weeks of the month were assumed to be representative of the entire month (Table 1). The simulated oyster population density (Fig. 21), spawning pattern (Fig. 22), and *P. marinus* infection intensity (Figs. 23,24) obtained for each of the four cases show that the oyster populations do consistently better as later weeks in the month are taken as the characteristic monthly value. Only in week 3, are the population attributes even remotely comparable to those observed using the weekly samples (cf. Figs. 18–20).

DISCUSSION

Food Supply

Food supply was estimated from measures of total particulate organic matter, phytoplankton biomass estimated from measures of chlorophyll *a*, and total labile organic matter estimated from a regression between measurements of chlorophyll *a* and total labile carbohydrate, lipid and protein. The third was clearly superior.

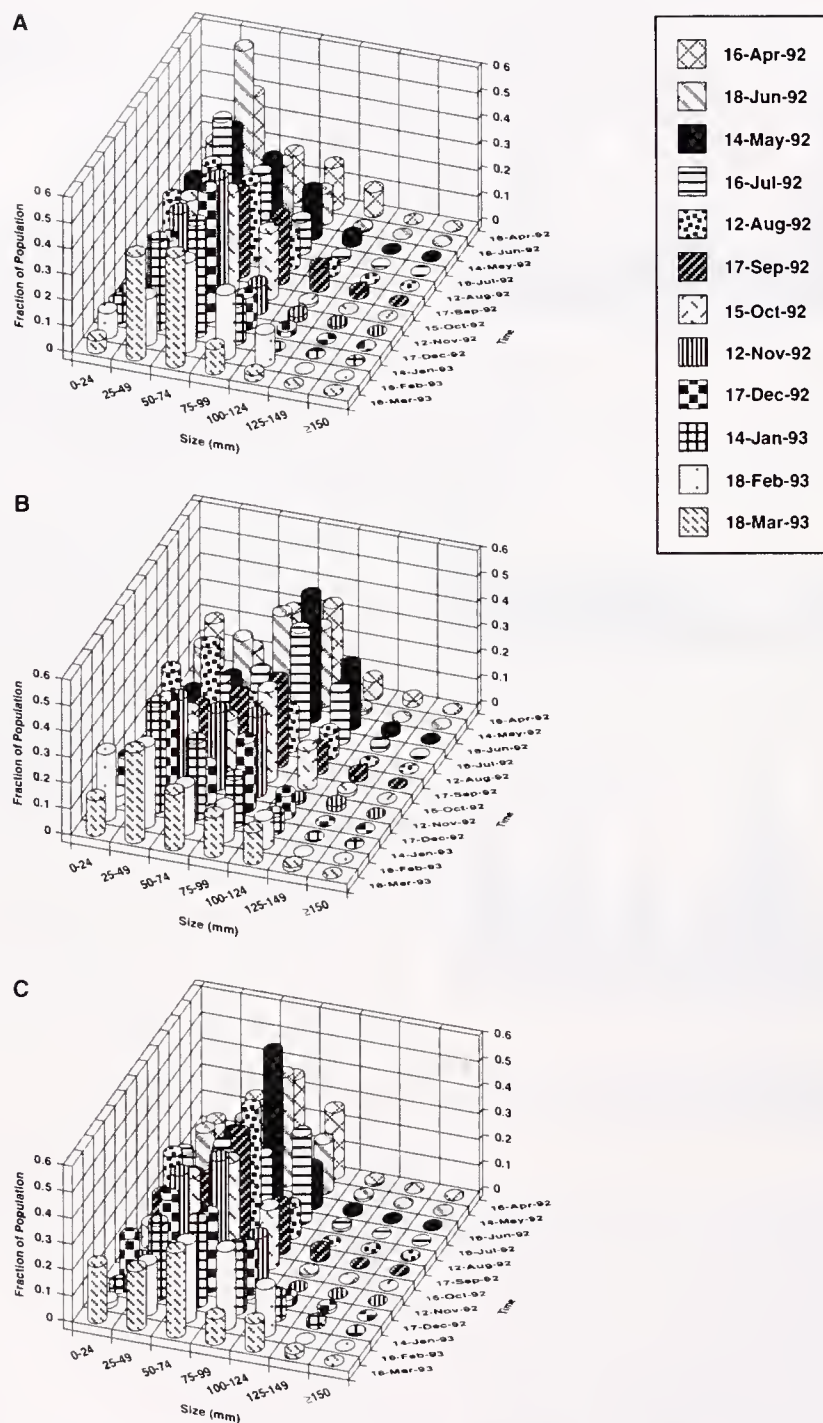


Figure 5. Size-frequency distribution of oysters obtained from the three sampling sites over the study period; (A) site 1, Savin Canal; (B) site 2, Bay Cocodrie; (C) site 3, Bay Tambour.

POM produced a simulated adult population that far exceeded the observed population in adult size. Total particulate organic matter is a poor measure of food supply unless a low assimilation efficiency is assumed. Langdon and Newell (1990) and Crosby et al. (1990) measured a low assimilation efficiency for refractive organic matter, and it is probable that POM from the Louisiana sites, like Galveston Bay (Wilson-Ormond et al. 1997), is mostly refractive. Assimilation efficiency was set at 75% in our

simulations. Thus, a lower assimilation efficiency might be used to provide simulated results more similar to field observations. However, as in Galveston Bay, the fraction of POM that is labile probably varies significantly during the year as phytoplankton biomass varies, and, in particular, the impact of the spring bloom on growth and reproduction in April to June could not be reproduced using POM. This spring/early summer event is crucial in determining population success (Hofmann et al. 1992). Thus, POM and

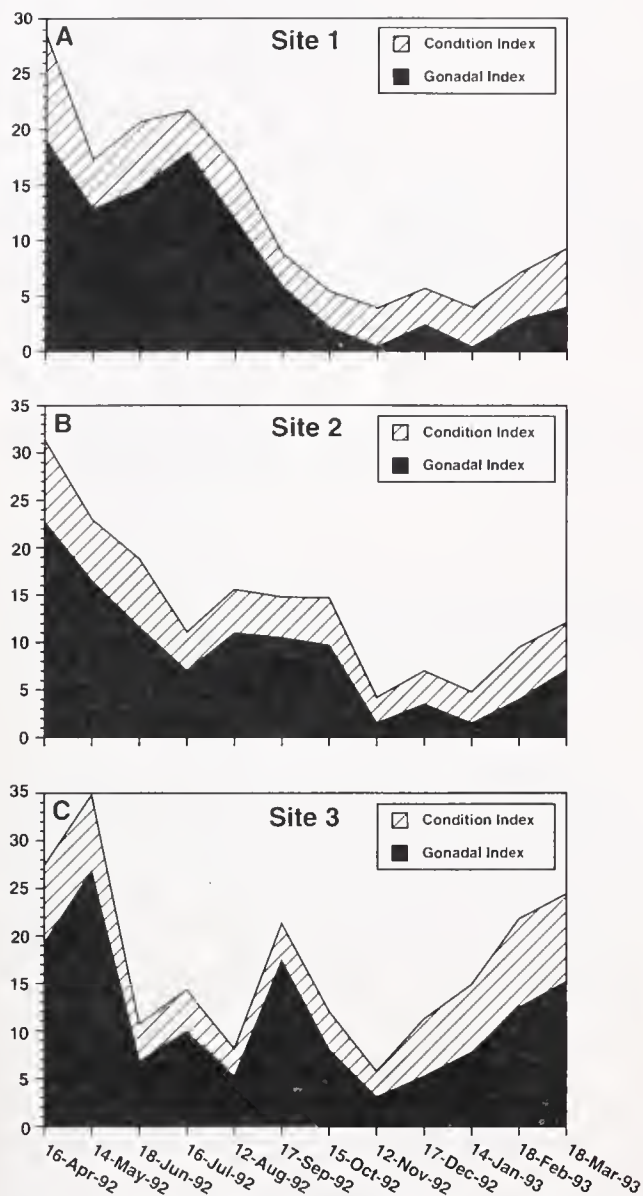


Figure 6. Gonadal index and condition index of oysters from the three sampling sites over the study period. Note that the y-axis scale differs between plots: (A) site 1, Savin Canal; (B) site 2, Bay Cocodrie; (C) site 3, Bay Tambour.

a low assimilation efficiency cannot produce a realistically simulated oyster population because the timing of the seasonal variation in food supply would be incorrect.

Chlorophyll *a* certainly measures an important component of oyster food (Berg and Newell 1986, Epifanio and Ewart 1977). Estimating food supply as phytoplankton biomass generates most important seasonal events in the oyster's life cycle. Growth, reproductive development, and spawning all occur at approximately the correct time in simulated populations, unlike that observed when POM was used. However, phytoplankton biomass simply does not provide an adequate food supply. Oysters remain too small, and reproductive development ends too early in the fall, coincident with the decline in phytoplankton standing stocks observed after July. Literature values will not support a higher as-

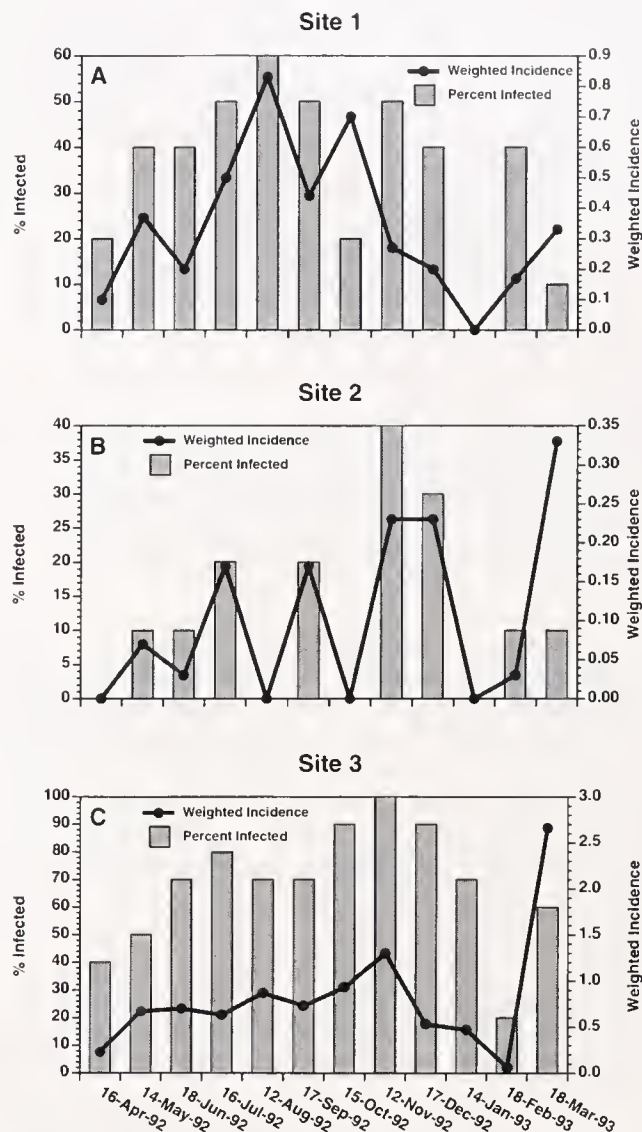


Figure 7. *Perkinsus marinus* prevalence and infection intensity for oysters from the three sampling sites over the study period; note that the y-axis scale differs between plots: (A) site 1, Savin Canal; (B) site 2, Bay Cocodrie; (C) site 3, Bay Tambour.

simulation efficiency (75% in these simulations) (Powell et al. 1992b, Tenore and Dunstan 1973, Valenti and Epifanio 1981). Thus, phytoplankton biomass is not a complete measure of oyster food.

Soniat et al. (1984) and Soniat and Ray (1985) demonstrated the importance of including a nonchlorophyll-explained food resource in the estimate of oyster food supply. They used the sum of three labile organic fractions: lipid, protein, and labile carbohydrate. In every test, this estimate of oyster food supply produced simulated oyster populations that most closely resembled those observed in the field. Similar results were obtained in simulations of oyster populations from Delaware Bay (Powell et al. 1997), Galveston Bay (Powell et al. 1994a), Gulf of St. Lawrence (unpubl. results), Chesapeake Bay (unpubl. results), and for a second oyster species, *C. gigas* (Kobayashi et al. 1997). The regression equation also has the interesting attribute of increasing oyster food

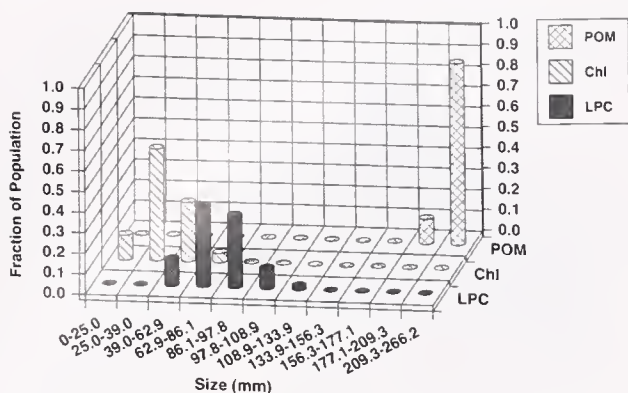


Figure 8. Comparison of simulated oyster population size frequencies at the end of year 6 for site 3 (Bay Tambour) using three different food resources: particulate organic matter (POM); phytoplankton biomass estimated directly from chlorophyll using the conversion factors of Widdows et al. (1979) and Parsons et al. (1961) (Chl); and food estimated from a regression between chlorophyll and total lipid, protein, and labile carbohydrate (LPC).

supply disproportionately in the fall and winter when phytoplankton stocks fall to seasonally low levels, as observed at the Louisiana sites. In the Gulf of Mexico, water temperatures remain high enough throughout the winter that oysters remain active for much of the time. Although a loss of condition is typically observed in larger animals (e.g., Fig. 6), growth may continue in juveniles. Phytoplankton biomass as the sole food resource produces an unacceptably large drop in biomass in simulated populations during the winter and does not produce the observed growth in juveniles. Appropriate winter population dynamics only occur if total labile organic matter is used.

Data and simulation both suggest that oysters depend upon a nonchlorophyll-explained food resource to supplement their diet and that this dependency is disproportionately important during the fall and winter in the Gulf of Mexico when phytoplankton stocks

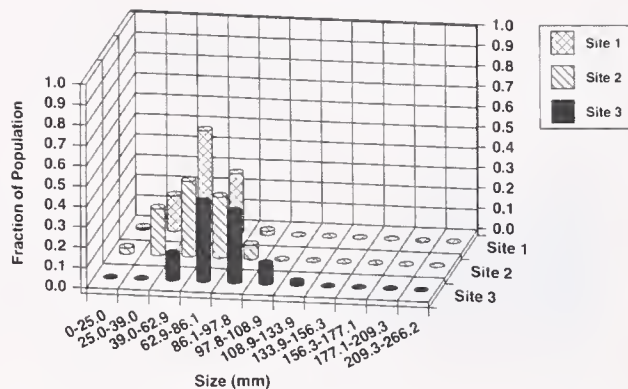


Figure 10. Comparison of the simulated size frequencies of oyster populations from the three sites at the end of year 6. Total lipid, protein, and labile carbohydrate was used as the food supply; (site 1) Savin Canal; (site 2) Bay Cocodrie; (site 3) Bay Tambour.

are low. Measures dependent upon direct conversion of chlorophyll to phytoplankton biomass, now the measures in routine use, do not adequately describe oyster food supply in the field, and may not adequately describe the seasonal variation in food supply any more than they do the absolute value.

Environmental and Biological Variables

Some years are wetter than others. Thus, sessile populations will experience differing salinity regimes as isohalines migrate up and down the estuary over the years. This year-to-year variability may provide a degree of stability, because some good years may permit populations to expand enough to survive lean years. In fact, oyster populations, in particular, may be stabilized by the year-to-year changes in environmental conditions that slow population expansion in the lean years and slow adult mortality in the better ones.

Simulated populations given the same environmental conditions year after year normally fall to extinction or increase in

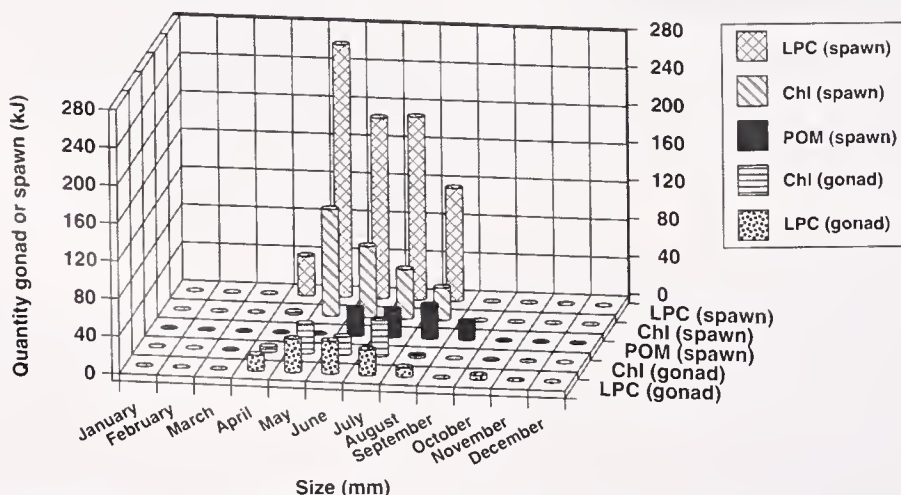


Figure 9. Comparison of simulated oyster population spawning pattern (monthly total of kJ of gametes spawned) and gonadal quantity (kJ gametic tissue). Spawning values represent the monthly total spawned by 10 oysters. Gonadal quantity represents the amount of gametic tissue present in 10 oysters on the last day of each month. Simulation results are for year 6 for site 3 (Bay Tambour) using three different food resources: particulate organic matter (POM); phytoplankton biomass estimated directly from chlorophyll using the conversion factors of Widdows et al. (1979) and Parsons et al. (1961) (Chl); and food estimated from a regression between chlorophyll and total lipid, protein, and labile carbohydrate (LPC).

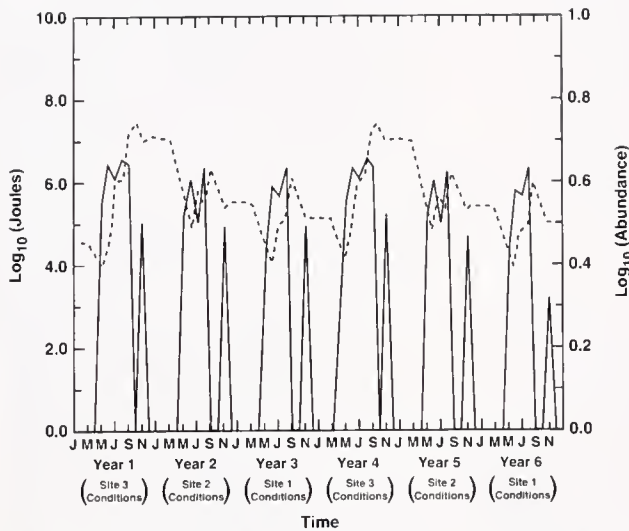


Figure 11. Comparison of oyster density [dashed line – $\log_{10}(\text{abundance})$] and reproductive effort defined as the amount of gametic tissue spawned [solid line in $\log_{10}(\text{joules})$] in a population exposed sequentially to the three environmental time series. Years 1 and 4 represent site 3 (Bay Tambour) conditions; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions.

abundance to abnormally high densities. Expansion and contraction are primarily a function of fecundity, larval survival, and postsettlement mortality. Distant brood stock supplying larvae may also play a significant role. Oyster population dynamics are characterized by thresholds that bound conditions yielding expansion or contraction and that prevent the existence of long-term equilib-

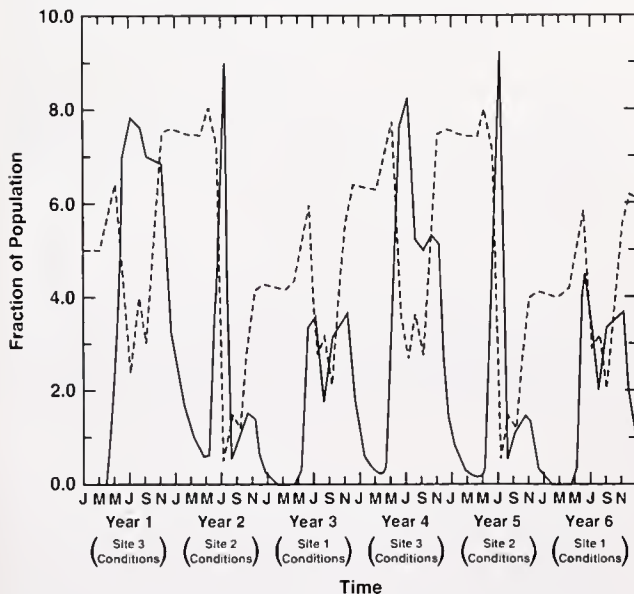


Figure 12. Comparison of *Perkinsus marinus* prevalence in a population exposed sequentially to the three environmental time series. Dashed line, the entire population. Solid line, the market-size portion of the population with prevalence calculated by assuming that oysters with $<4,000$ cells g dry wt^{-1} are recorded as uninfected. Years 1 and 4 represent site 3 (Bay Tambour) conditions; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions.

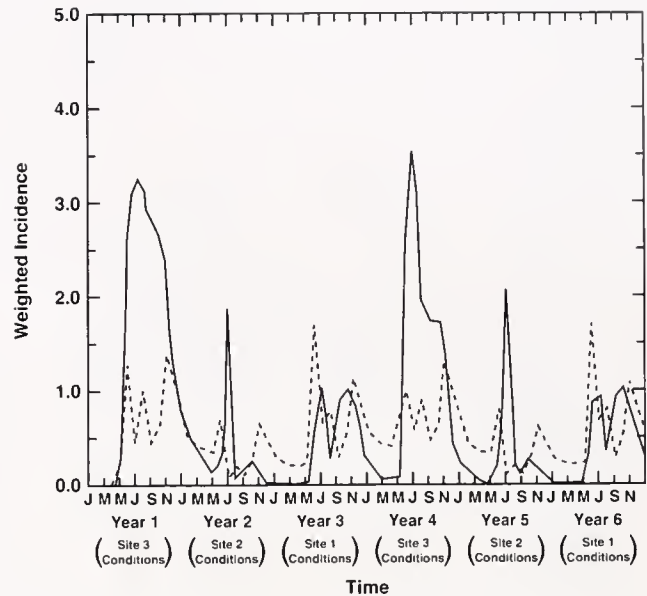


Figure 13. Comparison of *Perkinsus marinus* infection intensity in a population exposed sequentially to the three environmental time series for market-size oysters (≥ 76 mm) (solid line) and the entire population (dashed line). Years 1 and 4 represent site 3 (Bay Tambour) conditions; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions.

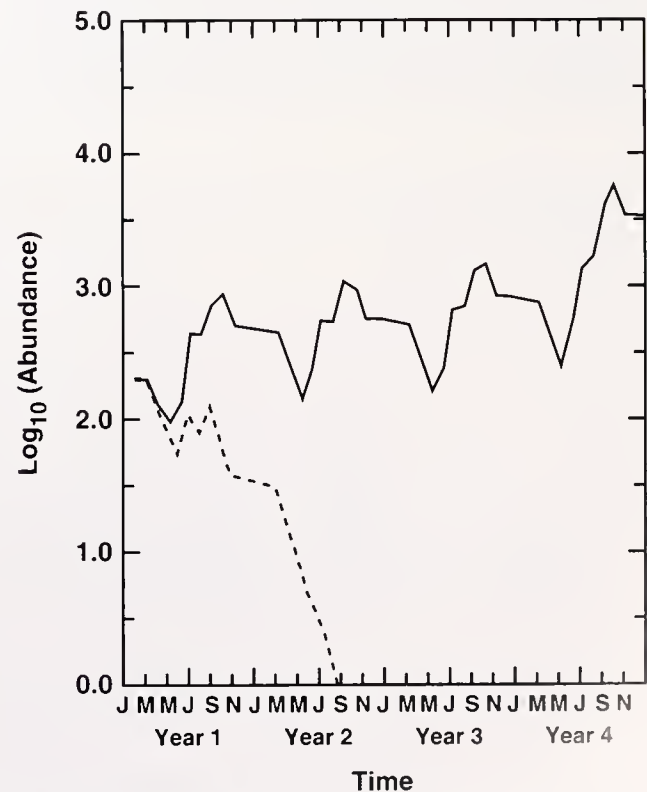


Figure 14. Comparison of simulated total population density [$\log_{10}(\text{abundance})$] of oysters at sites 1 (Savin Canal, dashed line) and 3 (Bay Tambour, solid line) exposed to the respective site-specific environmental time series for 4 continuous years, but with identical rates of larval survival and juvenile mortality at both sites.

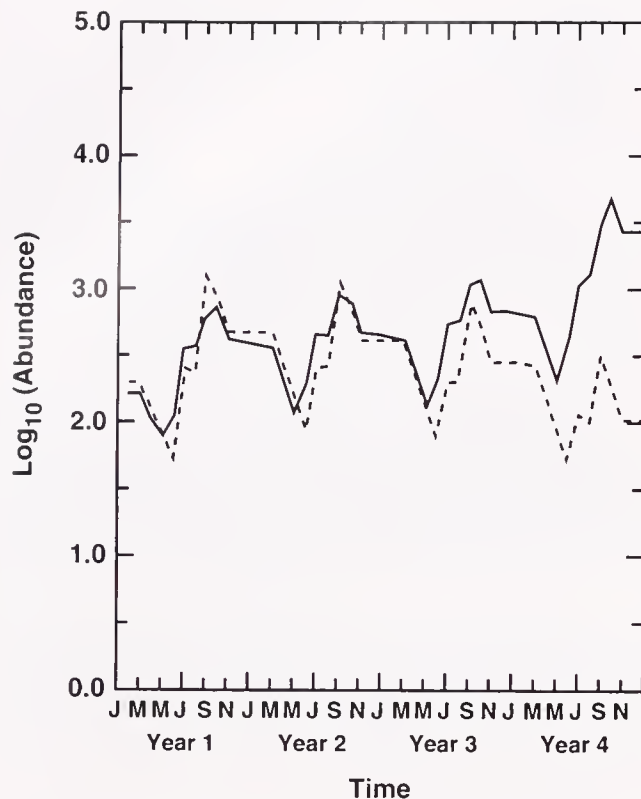


Figure 15. Comparison of simulated total population density [$\log_{10}(\text{abundance})$] of oysters at sites 1 (Savin Canal, dashed line) and 3 (Bay Tambour, solid line) exposed to the respective site-specific environmental time series for 4 continuous years, but with a higher rate of larval survivorship and a lower rate of juvenile mortality at site 1.

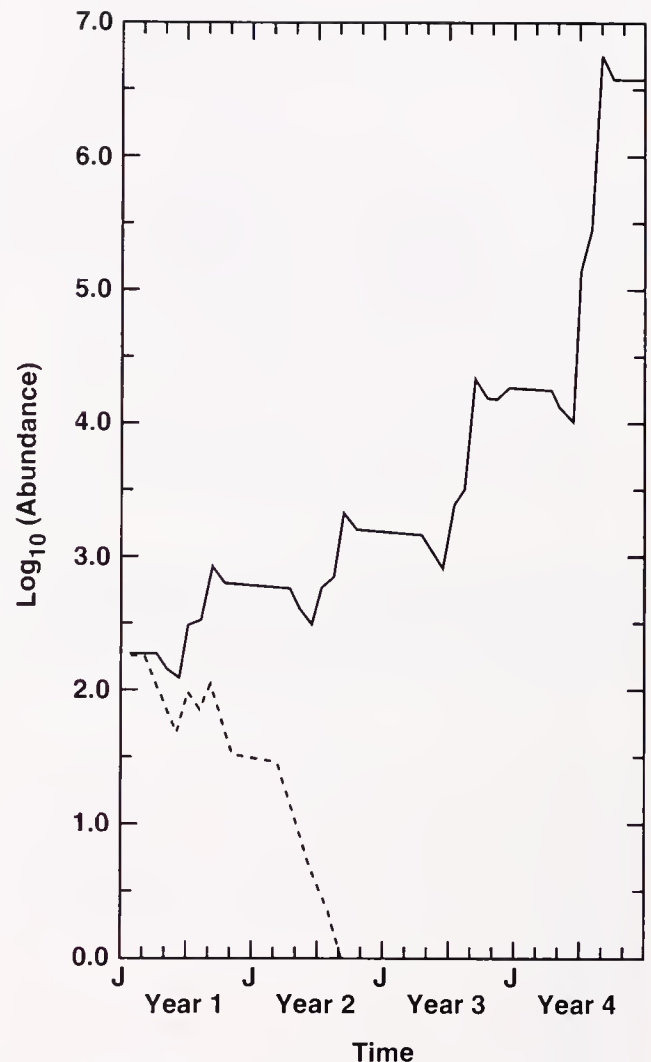


Figure 17. Comparison of simulated total population density [$\log_{10}(\text{abundance})$] of oysters at site 1 (Savin Canal) exposed to the site-specific environmental time series for 4 continuous years, but with differing rates of juvenile mortality; solid line, low juvenile mortality; dashed line, high juvenile mortality.

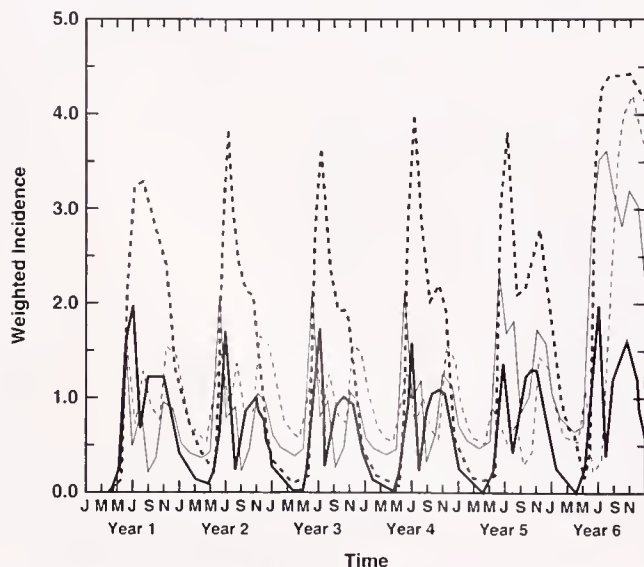


Figure 16. Comparison of simulated *Perkinsus marinus* infection intensity of oyster populations at sites 1 (Savin Canal) and 3 (Bay Tambour) exposed to the respective site-specific environmental time series for 6 continuous years, but with a higher rate of larval survivorship and a lower rate of juvenile mortality at site 1; solid line, site 1 (Savin Canal); dashed line, site 3 (Bay Tambour); thin line, entire population; thick line, market-size oysters.

ria under a constant environmental regime, as shown in our simulations. The inference from these simulations is that oyster populations are normally either expanding or contracting and that the year-to-year variations in environment are overwhelmingly important in restricting the time allotted to each process. It follows that multiyear sampling of environmental variables is extremely important.

The short-term effect of such environmental variables as the effect of an increase in temperature on the rate of *P. marinus* cell division is relatively easy to simulate. However, many population attributes integrate a longer history of environmental change. Any population is a product of the time-history of the environmental and biological processes that have occurred over a number of generations, and these processes will change seasonally and from year to year. Thus, a detailed comparison between simulation results and field observations will always yield inconsistencies because of the limited time span of the environmental data and, because the repetition of environmental data from one year to the next is unlikely to mimic field conditions. In particular, simula-

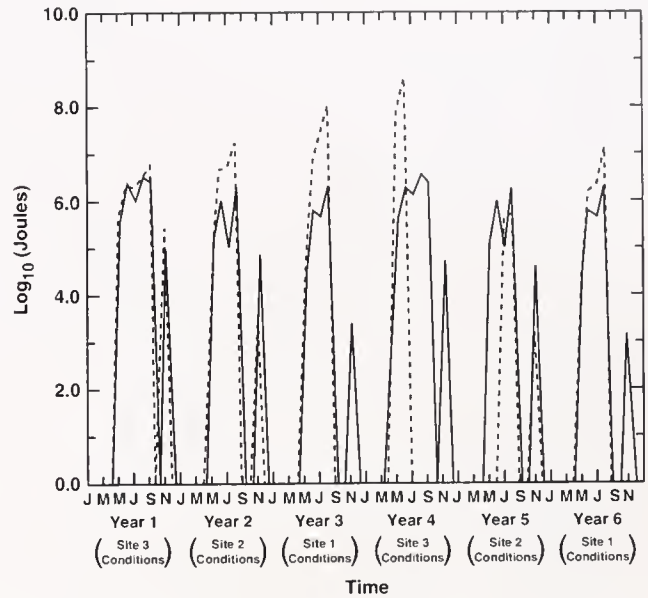


Figure 18. Comparison of simulated oyster densities [$\log_{10}(\text{abundance})$] in a population exposed sequentially to the three environmental time series. Years 1 and 4 represent site 3 (Bay Tambour) conditions; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions. Solid line, results using the weekly environmental measurements with daily values obtained by linear interpolation between measurements; dashed line, results using the average of four weekly samplings to define the monthly value and daily values obtained by linear interpolation between the 15th of each month.

To examine the influence of multiyear time series and relative influence of the most recent year in the time series, we appended the environmental variables for the three sites so that the environmental conditions of site 3 were followed by those for site 2 and then those for site 1. For brevity, we show results only for this sequence, which is a high-low-moderate salinity time series. The results emphasize the importance of multiyear environmental time series in describing the time-history of a relatively long-lived species. Only with a multiyear time series introducing interannual environmental variation did the simulated populations at each site conform to field observations. The results also emphasize that the most recent year exerts the strongest impact, as anticipated, par-

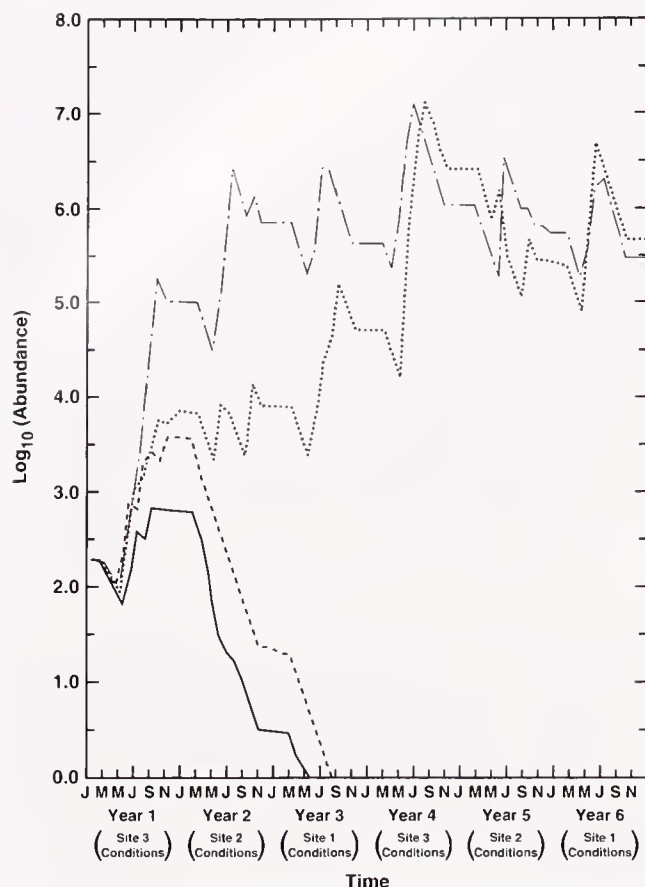


Figure 21. Comparison of simulated oyster densities [$\log_{10}(\text{abundance})$] in a population exposed sequentially to the three environmental time series. Years 1 and 4 represent site 3 (Bay Tamhoure) conditions; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions. Solid line, results using the environmental measurements for week 1 to define the value on the 15th of each month. Dashed line, analogous results using the environmental measurements for week 2. Dotted line, analogous results using the environmental measurements for week 3. Dot-dashed line, analogous results using the environmental measurements for week 4.

ticularly in such environmentally sensitive variables as spawning and *P. marinus* infection intensity, but that the time-history modulates the effect of the most recent environmental signal.

The simulations reveal a distinct gradient between sites 3, 1, and 2 in the susceptibility of populations to local extinction. In particular, site 3 is better able to support its own population through reproduction. The simulations show the importance of the environmental conditions measured at site 3 and suggest that low salinity populations in the Terrebonne Basin are sustained either by outside larval settlement or by year-to-year variability in environmental conditions, providing that good years occur frequently enough. Achieving a persistent population at site 2 requires either higher larval survival or lower postsettlement mortality than at site 3, if the salinity regime remains constant. Although the present field data cannot distinguish the two alternatives, because the number of predators is unknown, it is likely that site 2 and probably site 1 require an external source of larvae during consecutive low-salinity years to limit the chance of local extinction.

It is interesting that a reasonable simulation is achieved by sequentially juxtaposing the environmental time series, while

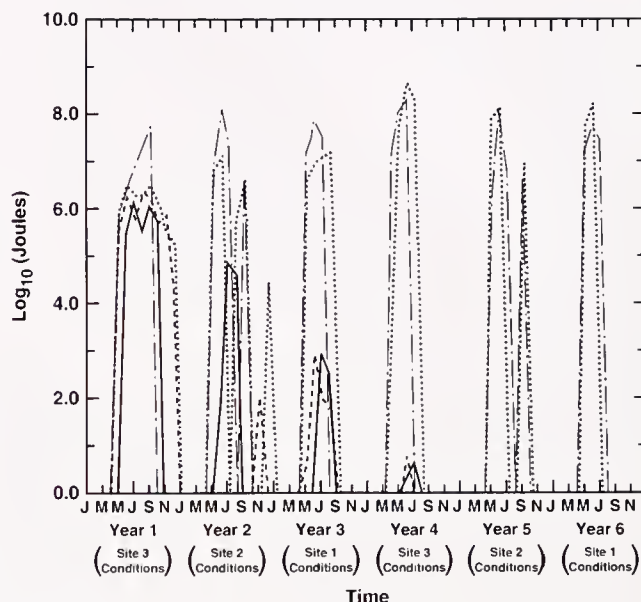


Figure 22. Comparison of spawning [$\log_{10}(\text{joules of gametes spawned})$] in a population exposed sequentially to the three environmental time series. Years 1 and 4 represent site 3 (Bay Tamhoure) time series; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions. Solid line, results using the environmental measurements for week 1 to define the value on the 15th of each month. Dashed line, analogous results using the environmental measurements for week 2. Dotted line, analogous results using the environmental measurements for week 3. Dot-dashed line, analogous results using the environmental measurements for week 4.

maintaining the same biological characteristics for the oyster population (e.g., rates of larval survival and juvenile mortality). Population persistence and, therefore, adequate simulations can be created by persistent shifts in the environment or by biological gradients in larval settlement, juvenile mortality, etc. Very likely, sequential shifts in environmental regime are also associated with changes in predator abundance and rates of larval recruitment; however, the simulations indicate that simple sequential shifts in environmental variables adequately explain the observed population attributes in these Terrebonne Basin sites. This indicates, for example, that fecundity during good years (= site 3 conditions) may be sufficient to permit population persistence during bad years (= site 2 conditions) in this area. Whether populations persist without an external larval supply will depend upon the relative frequency of these good and bad years. The suggestion from these simulations is that population dynamics at these sites is most influenced by the time-history of environmental change and less influenced by variations in predator abundance and larval survivorship.

However, these populations are heavily influenced by disease and, on occasion, by low-salinity mortality, which adds an important mortality gradient at these sites and in the simulations. Non-diseased populations or populations where disease is a minor contributor to mortality would certainly behave differently. Furthermore, all three sites are in areas where oyster drills are probably not a major source of mortality; whereas, crabs are. Crab densities are less influenced by environmental gradients (Powell et al. 1997), and, thus, rates of crab-induced mortality may be similar among all three sites. Therefore, the data and simulations do not completely discount the importance of determining predator abun-

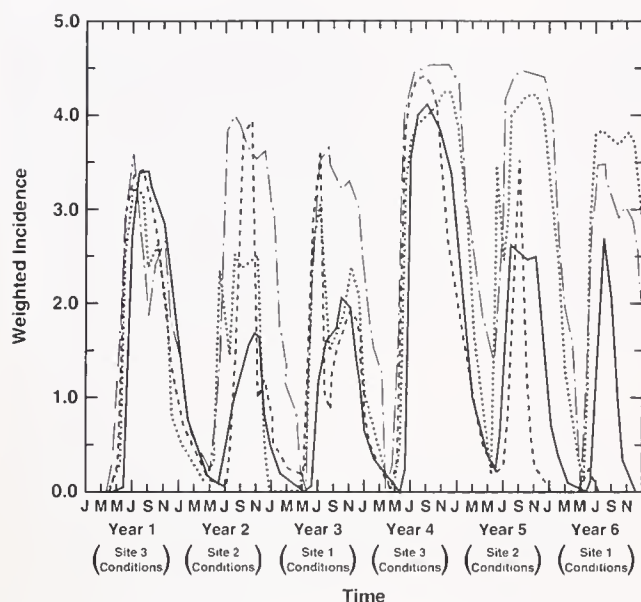


Figure 23. Comparison of *Perkinsus marinus* infection intensity in market-size oysters in a population exposed sequentially to the three environmental conditions. Years 1 and 4 represent site 3 (Bay Tambour) time series; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions. Solid line, results using the environmental measurements for week 1 to define the value on the 15th of each month. Dashed line, analogous results using the environmental measurements for week 2. Dotted line, analogous results using the environmental measurements for week 3. Dot-dashed line, analogous results using the environmental measurements for week 4.

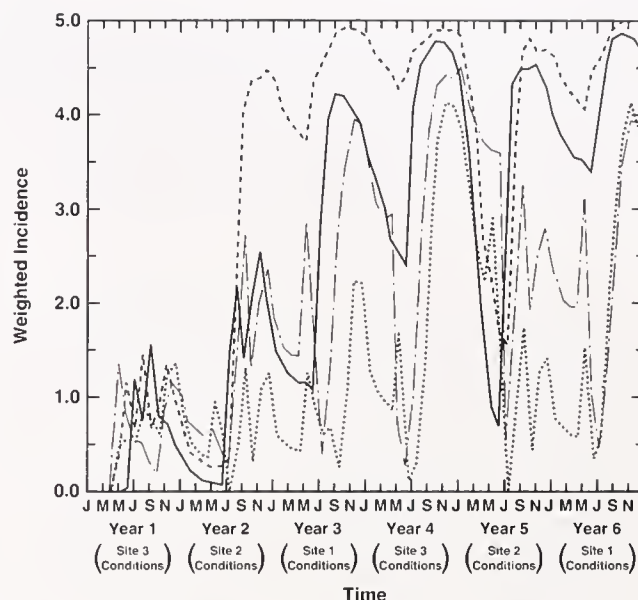


Figure 24. Comparison of *Perkinsus marinus* infection intensity in the entire population of oysters exposed sequentially to the three environmental conditions. Years 1 and 4 represent site 3 (Bay Tambour) time series; years 2 and 5, site 2 (Bay Cocodrie) conditions; years 4 and 6, site 1 (Savin Canal) conditions. Solid line, results using the environmental measurements for week 1 to define the value on the 15th of each month. Dashed line, analogous results using the environmental measurements for week 2. Dotted line, analogous results using the environmental measurements for week 3. Dot-dashed line, analogous results using the environmental measurements for week 4.

dances in understanding the mechanisms producing the observed oyster population attributes, but they do emphasize the necessity of a long-term series of environmental conditions.

Our results indicate the power of combining field research and simulation modeling in understanding the processes producing observed population structure. In this case, the combination of the two has distinguished the potential importance of environmental shifts and biological interactions and suggested further research to test these hypotheses. In addition, the combination has permitted estimation of the sensitivity of these populations to local extinction and the likely mechanisms. Of course, many of these inferences could have been obtained by examining a very long-term dataset for these populations, but resources normally preclude the long-term collection of the intensive dataset on population attributes required for observational inference.

The Importance of Adequate Sampling

Sampling frequency is often determined by funding. In planning a field research program on oyster population dynamics, the immediate question is: how intensive must sampling be to permit an adequate evaluation of observed population attributes or to produce adequate model simulations? A comparison of monthly versus weekly data in the time series clearly shows that field observations are not adequately simulated using monthly environmental data, at least for these Terrebonne Basin sites. Whether even shorter time-scale sampling would provide a further improvement over weekly sampling is untested, but has been assumed in recent modeling studies in Galveston Bay (Powell et al. 1994a) and Delaware Bay (Powell et al. 1997), where hourly or daily envi-

ronmental data have been used. Regardless, obviously, weekly sampling provides a quantum leap in accuracy over monthly data in simulations and, presumably, a fundamental improvement in the ability to evaluate the mechanisms determining population structure in natural populations.

The Importance of Sampling Time

Very little consideration has been given to the timing of sampling during the month. In the spring, week 1 is routinely colder than week 4. In the fall, the opposite is true. However, much of the population attributes of the oyster population for the year are established in the crucial period of the spring when temperature is rising, as is food supply (Hofmann et al. 1992). Colder temperatures do not permit oysters to take advantage of the increase in food supply, and, on the average, more food is available in the later weeks of each spring month. Hofmann et al. (1992) discussed the crucial nature of the timing of the spring bloom and the rise in temperature in the spring. The opposite conditions in the fall are less important, because oyster metabolism slows down, and food supplies are normally low. Thus, sampling time and frequency are important, particularly in the spring, and, as important, is the recognition that explicit sampling time must be retained in all analyses rather than permitting a monthly sampling to pertain simply to the entire month.

In this study, a sampling fell no more than 10 days from the middle of the month in the most extreme case; however, simulation accuracy was substantially degraded if these values were assumed indicative of the entire month. The simulations emphasize the importance of a change in environmental signal over a few weeks

or less during the spring and early summer. The simulations also emphasize that the temptation of applying monthly measurements at one time to other monthly data obtained at an independent time must be eschewed. For example, comparing monthly mean environmental conditions with a single event stock assessment a week or more different in time will likely produce inaccurate comparisons that fail to clarify the reasons for population success or failure.

CONCLUSIONS

The comparison of direct observations of oyster populations through field sampling and the population attributes obtained from simulation modeling emphasizes the importance of combined field and modeling studies to develop hypotheses about how environment and biology influence population attributes and to design field sampling programs. Simulations only approached field observations with weekly sampling and with the correct food resource estimated. Simulations also only approached field observations when the assumption was made that oyster populations are chronically in disequilibrium with the environment so that year-to-year changes in environment contribute substantially to observed population attributes. This latter finding, of course, is well

documented in a number of studies of time series in oyster populations (Ulanowicz et al. 1980, Allen and Turner 1989). Finally, in these simulations, the influence of environment and *Perkinsus marinus* disease seemingly overwhelmed the influence of year-to-year changes in predator abundance or larval survival in establishing the population attributes in the field at our Terrebonne Basin sites. At least in this area of the Gulf of Mexico, weekly sampling of environmental variables would seem to be more important than a more detailed monthly enumeration of predator abundances, although the latter would certainly be beneficial. Probably, this finding is typical of the Gulf coast, where *P. marinus* is prevalent, but it may not apply throughout the range of the American oyster.

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FEASIBILITY OF BIOLOGICAL CONTROL OF ALGAL FOULING IN INTERTIDAL OYSTER CULTURE USING PERIWINKLES

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ABSTRACT The effectiveness of the use of periwinkles (*Littorina littorea* L.) to control the fouling algae in oyster bags was tested at the Eo estuary in northwest Spain. Growth was higher for Japanese oysters (*Crassostrea gigas* Thunberg) when cultured with than without periwinkles (87.74 g vs 60.07 g of mean wet weight). This biological control of fouling shortens the growth period and thus reduces production cost. The system appears to be one of the most effective and most environmental friendly methods of limiting the negative effect of the seaweeds in intertidal oyster culture.

KEY WORDS: Biofouling, Japanese oyster, periwinkle, Eo estuary

INTRODUCTION

Biofouling is a major problem currently facing marine technology. In the marine environment, materials submerged experience a series of discrete physical, chemical, and biological events which result in the formation of a complex layer of attached organisms known as biofouling (Abarzua and Jakubowski 1995). These organisms likely reduce water flow through bivalve culture enclosures and ultimately, the flux of food particles to the bivalves. Also, many fouling organisms are suspension feeders and may compete with cultured bivalves for food (Lodeiros and Himmelman 1996). Therefore, in oyster culture, intensive competition for living space and food develops among oysters and fouling organisms (Arakawa 1990).

A number of physical (e.g., heat, exposure to sun, boiling, concentrated brine bath) and chemical (e.g., insecticides, herbicides) techniques have been developed to limit biofouling in oyster culture (Arakawa 1980, 1990, Marteil 1979). Although these methods are effective, they increase labor costs during growout. Therefore, an efficient, inexpensive means of ensuring maximum water flow to oysters is greatly needed (Enright et al. 1983).

Compared with chemical methods of eradicating fouling (e.g., DDT, chlorides), biological control is less likely to produce side effects such as pollution and it holds promise for the future of the fisheries industry (Arakawa 1990). Unfortunately, biological control of fouling is an undeveloped field in shellfish culture, even though several studies indicate it is efficient (e.g., Hidu et al. 1981, Enright et al. 1983, Minchin and Duggan 1989). In addition, there are no long-term studies evaluating the economical and biological effects of the use of techniques to control fouling.

The objective of this study was to determine the economical and biological suitability of using periwinkles to control biofouling, by comparing the growth and survival of oyster sets with and without periwinkle. As algae are the most important fouling organisms in our area, we chose in the natural environment the periwinkle as the control agent, a major algal grazer.

MATERIALS AND METHODS

Two sets of Japanese oysters, *Crassostrea gigas* (163,000 oysters, 1.02 g mean live weight) were placed in bags in the intertidal zone of the Eo estuary in May 1995. In one set the oysters were maintained without periwinkles as a control and in the other, periwinkles were added to the bags. In both sets, the density of oysters was about 240 oysters per bag.

Periwinkles were collected in the Eo estuary and placed in the bags with oysters the next day when oysters were planted. Density was about 250 periwinkles per bag and 3.2 g mean live weight.

Both sets were cultured for 18 months in standard oyster bags (1 × 0.5 m, 6 mm diameter) which were placed on metal tables (0.6 m high and 3 m in length). All bags were turned over each spring and autumn to prevent the oysters from attaching to the bag and to reduce algae on the upper side of the bags. To estimate mortality, we opened 10 oyster bags per set and determined the percentage of live oysters per bag. Forty oysters from each set were randomly collected from these 10 bags every 3 months. Oysters were washed and then weighed to the nearest 0.1 g.

Weight and survival data were log (\log_{10}) and percentage transformed, respectively. Then they were tested for normality (Lilliefors test, $p > .1$) and homogeneity of variance (Bartlett test, $p > .05$). After that, we employed one-way analysis of variance (ANOVA) to compare differences in weight between the two treatments on each date. ANOVA was also used to study survival differences at the end of the experiment. Weight frequency distributions of oysters in the two treatments at the end of the experiment were compared using a Kolmogorov-Smirnov test ($p > .05$).

RESULTS AND DISCUSSION

In the Eo estuary the most important fouling organisms in oyster culture are algae which colonize the upper sides of bags (mainly *Enteromorpha compressa* Greville, *E. prolifera* Müller, *Ulva* sp., *Ceramium nodulosum* Ducluzau, and *Polysiphonia* sp.)

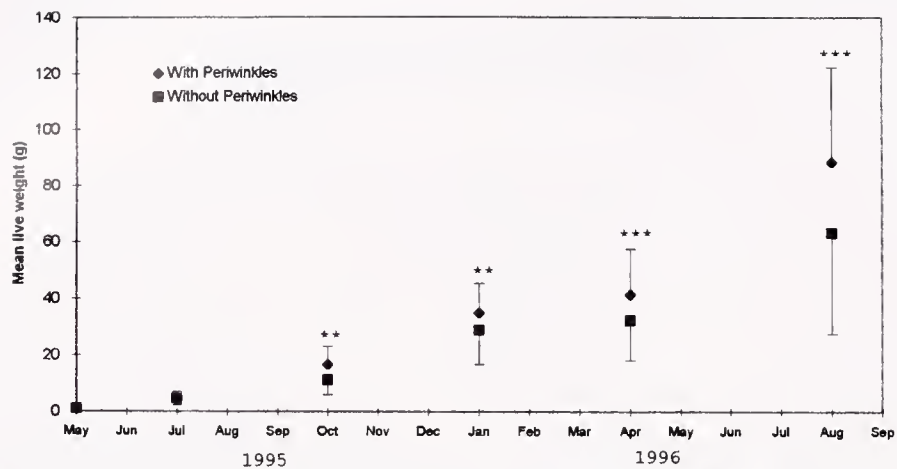


Figure 1. Mean live weight of Japanese oysters (± 1 SD) in treatments with and without periwinkles from March 1995 to November 1996.

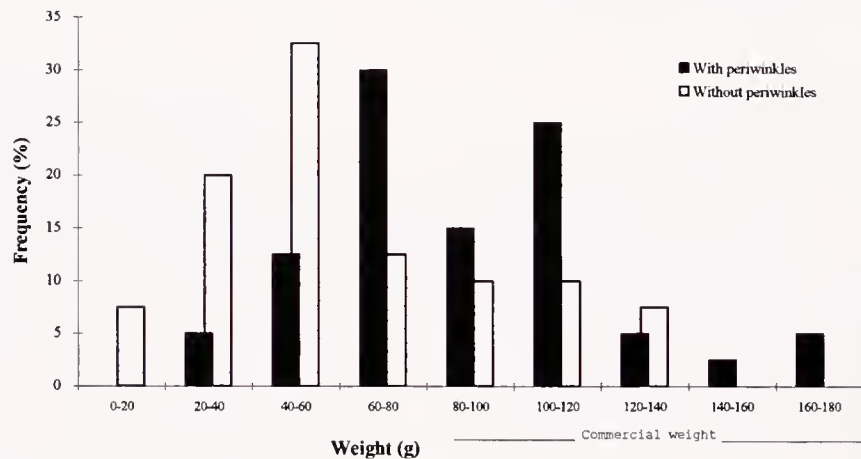


Figure 2. Individual weight frequency distribution for oysters cultured with and without periwinkles at the end of the experiment.

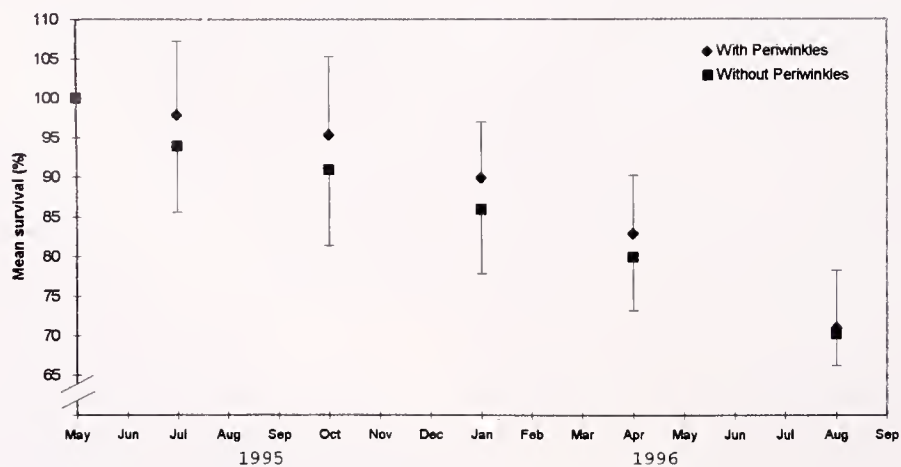


Figure 3. Average survival (± 1 SD) of oysters with and without periwinkles.

and tunicates and sponges which colonize the lower sides. The latter organisms accounted for less than 10% of total biofouling. The grazing of the periwinkle reduced algae, likely increasing the flow of water and food particles into the bags, thus increasing the growth of oysters, but, as they do not prey or compete with oysters, survival was similar in both treatments. So mortality was similar ($p > .1$) whether periwinkles were present or not (Fig. 3).

The mean live mass of oysters was significantly higher in the treatment with than without periwinkles ($p < .001$) (Fig. 1). More importantly, the percentage of oysters that reached commercial size (>80 g) in treatment with periwinkles was twice that without periwinkles (47.6% vs 21.17%) (Fig. 2) ($p < .001$). The growth of oysters without periwinkles was comparable to that obtained in previous studies in the intertidal zone using stocking densities similar to ours (Berthome et al. 1986, Guerra et al. 1987), whereas growth of oysters in bags with periwinkles was higher. Periwinkles showed a reduced growth (from 3.2 g mean live weight to 3.8 g), and although they could be sold for additional revenue, labor costs would also be much increased as it is very difficult to select periwinkles from the oysters, empty shells, and fouling organisms.

Extrapolation of our growth and survival data until all oysters were of harvestable size indicate that the main advantage of using periwinkles is that the cycle of culture can be reduced from 4 to 3 years, which considerably reduces the production costs (Table 1). Labor costs were reduced as less hours were needed to select the commercial-sized oysters. Nevertheless, it is still necessary to turn over the bags as this operation allows homogeneous growth resulting from the redistribution of oysters in the bags.

In conclusion, the results presented here suggest that the use of periwinkles in algae control has a great potential as a fouling control method, which could be easily applied in oyster culture.

TABLE 1.

Estimated parameters for complete cycle of culture until all oysters reach commercial size.

	With Periwinkles	Without Periwinkles
Culture time (years)	3	4
Harvest of commercial-sized oyster (%)		
First year	0	0
Second	78	47
Third	22	48
Forth	0	5
Sales (%)	100	100
Variables costs (%)	56	56.06
Seed	8.35	8.35
Periwinkles	1.17	0
Labor	22.62	23.85
Commercialization	23.86	23.86
Fixed costs (%)	16.44	21.75
Profit (%)	27.56	22.19

We extrapolated growth and survival until the end of culture using the model of Fernández & Cigarría (1997). Variable costs include labor, purchase of seed and periwinkles, and commercialization. Fixed costs mainly include amortization of bags, tables, etc.

However, this appraisal is based on one small experiment studied only at one site of culture, and should be regarded as a preliminary result. More data about growth, survival and optimum density of periwinkles into the bags, influence of the site of culture, tidal level, etc. would be necessary to optimize this method of oyster culture.

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CULTURED OYSTERS, *CRASSOSTREA VIRGINICA*, GENETICALLY SELECTED FOR FAST GROWTH IN THE DAMARISCOTTA RIVER, MAINE, ARE RESISTANT TO MORTALITY CAUSED BY JUVENILE OYSTER DISEASE (JOD)

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ABSTRACT Growth and mortality of hatchery-produced juvenile oysters, *Crassostrea virginica*, selected for fast growth were compared with unselected, wild oysters at sites in Maine (Damariscotta River) and Massachusetts (North Bay) where Juvenile Oyster Disease (JOD) is enzootic. Over the course of the study, JOD occurred primarily in Maine, even though prevailing temperature and salinity at both sites were conducive for JOD development. From July to November 1996, mean shell height of selected oysters was greater than that of wild oysters at both sites. At the end of the study, mean shell height of both selected and wild oysters was greater in Massachusetts than in Maine. Mean cumulative mortality of both groups was greater in Maine than Massachusetts. Only in Maine was mean cumulative mortality of wild oysters greater than that of selected oysters. Differences in growth and mortality of oysters between sites were due primarily to the differential timing and severity of JOD occurrence. In addition, the difference in survival between selected and wild oysters in Maine was not related to differences in size between groups at the time of initial exposure to JOD. Thus, we conclude that occurrences of JOD are site specific (not dependent on source of seed), and that under challenge from JOD, selected oysters not only grow faster than wild (unselected) oysters, but exhibit a genetically based tolerance of this disease.

KEY WORDS: *Crassostrea virginica*, selection, Juvenile Oyster Disease, growth, mortality

INTRODUCTION

The culture of oysters, *Crassostrea virginica* (Gmelin 1791) in the northeastern United States has been severely impacted since 1988 by mortalities of hatchery-produced seed caused by Juvenile Oyster Disease (JOD). Signs of the JOD syndrome include reduced growth, shell deformation (cupping of left valve), tissue emaciation, mortality approaching 100%, and heavy conchiolin deposits on inner valve surfaces (Bricelj et al. 1992, Davis and Barber 1994, Lewis et al. 1996). JOD is site- and species-specific. Certain locations in Maine, Massachusetts, and New York have chronic disease outbreaks but others do not. Only hatchery-produced *C. virginica* is affected by JOD (Bricelj et al. 1992); *Ostrea edulis* cultured in close proximity to *C. virginica* does not contract the disease (Crosby and Barber unpublished data). Although a specific causative agent has yet to be identified, Lewis et al. (1996) demonstrated that JOD is transmissible and that greatest transmission occurs at water temperatures of 22–26°C and salinities of 18–30 ppt.

Mortality caused by JOD is inversely related to size, with cohorts having a mean shell height of <25 mm at the time of initial JOD occurrence, experiencing the greatest mortality (Bricelj et al. 1992, Davis and Barber 1994). JOD occurs only during summer months, primarily at water temperatures >20°C (Bricelj et al. 1992). Seasonal aspects of JOD were investigated by Barber et al. (1996) who produced eight cohorts of oysters at 2-wk intervals throughout a growing season and found that mortality was dependent on the timing of placement in the field; cohorts deployed before the end of May and after mid-August had <20% mortality, but all other cohorts had mortalities >65%. The cohort that was deployed in May was exposed to JOD, but because it had reached a mean size of >25 mm at the time of JOD occurrence, suffered minimal mortality; the cohort deployed in mid-August also experienced little effect from JOD. It was concluded that etiological agent(s) of JOD are only present or active seasonally, but that within the “window of infection,” signs of JOD (reduction in

growth, valve cupping, mortality) were not manifested until 3–4 wk after placement in the field (Barber et al. 1996).

Knowledge of the timing of JOD outbreaks and the relationship between mortality and oyster size has led to disease management techniques which include producing seed early in the spring or in the fall as well as using nursery sites where JOD does not occur (Barber et al. 1996). Additional benefit can be achieved by utilizing oysters selected for fast growth. A program of genetic selection for fast growth of *C. virginica* was initiated at the University of Maine in 1986 in conjunction with the state’s expanding oyster culture industry (Hawes et al. 1989). In general, “selected” sub-lines [parents from the largest 20% (total weight) of the previous generation of selected oysters] have outperformed “control” sub-lines (parents from the entire weight distribution of the previous generation of control oysters), both in terms of growth and survival in the Damariscotta River, Maine, where JOD is endemic (Davis et al. 1990, 1991, Davis and Barber unpublished data). Thus, though these oysters have been consciously selected for fast growth, they have also, by default, been selected for resistance to JOD mortality in recent years. To assess the effectiveness of this selection process to date, we compared growth and survival of a selected cohort with that of an unselected, JOD-susceptible cohort at two locations where JOD is enzootic.

METHODS

Broodstock oysters used to produce the “selected” cohort were the largest 20% (total weight) of surviving Flowers F₂ selected oysters produced in 1994. Parents of the unselected “wild” oysters (not previously exposed to JOD) came from natural beds in the Piscataqua River, Maine. Broodstocks were conditioned separately beginning in February 1996. Both groups were induced to spawn using thermal shock on 23 May, 1996. The number of parents contributing gametes was 13 males and 5 females for the selected group and 8 males and 12 females for the wild group.

Larvae were reared in 2000 l conical tanks containing filtered

(10 μ m) water from the Damariscotta River, which was replaced every other day. Temperature in the tanks was maintained at 22–24°C and food (mixed diet of *Isochrysis galbana*, *Tetraselmis* sp., and *Thalassiosira pseudonana*) was added twice per day. Eyed larvae were collected and placed into a setting tray containing mini-cultch (crushed oyster shell) on a screen. After the settlement period (48 h), spat were placed in downwellers and fed a diet similar to that given larvae.

Spat (2–3 mm shell height) were placed at two sites: Damariscotta River, Newcastle, Maine (lat 44°01'N; long 69°32'W) and North Bay, Osterville, Massachusetts (lat 41°37'N; long 70°26'W) on 4 and 5 July 1996, respectively. Five replicate trays, each containing 500+ oysters (stocking density = 2,000 oysters/m²), were deployed for both selected and wild groups. Oysters at both locations were sampled regularly between July and November 1996. On each sampling date, shell height measurements were obtained from a subsample of 50 individuals per replicate using either image analysis (from photographs) for small (<5 mm) oysters or digital calipers for larger oysters (>5 mm). The averages obtained from each of the five replicates were used to calculate mean shell height for that date. All individuals were checked for general health and all dead oysters were counted and removed from trays. Cumulative mortality was calculated as the sum of instantaneous mortality rates (\log_e finite rate). Finite mortality rate was defined as the number of oysters that died during each time (sampling) interval divided by the number of oysters alive at the beginning of the interval (Krebs 1972). Mean cumulative mortality was calculated for each group on each date using the five replicate cumulative mortalities.

At the North Bay site, salinity was recorded on each sampling date with a refractometer, and temperature was recorded every 3 h with a datalogger (Onset StowAway). At the Damariscotta River site, salinity was determined biweekly with a YSI salinometer and temperature was recorded every 6 h with a thermograph (Ryan RTM 2000).

For each sampling date, mean shell heights and cumulative mortalities (arcsin transformed) of both selected and wild groups were statistically compared between sites using a one-way ANOVA and Duncan's Multi-Range post hoc test and within sites using a *t*-test (DataMost 1995).

RESULTS

In the Damariscotta River, Maine, growth of both groups was rapid between 4 July and 16 August, but slower thereafter (Fig. 1). Mean shell height in the wild group on 16 August (16.5 mm) increased only slightly to a final mean shell height of 18.7 (± 2.8 SD) mm on 21 November. In contrast, the selected group continued to grow until 12 September, reaching a mean shell height of 33.5 (± 0.5 SD) mm on 21 November. Mean shell height of selected oysters was significantly greater than that of wild oysters on all sampling dates ($p \leq .0002$).

Selected oysters also grew faster than wild oysters in North Bay, Massachusetts (Fig. 1). Compared with the Damariscotta River site, however, growth of both groups was more continuous and occurred over a longer period of time (5 July to 17 October). On 14 November, mean shell height of the wild group was 27.7 (± 1.3 SD) mm and that of the selected group was 38.9 (± 0.5 SD) mm. Differences in mean shell height between selected and wild groups were significant on all sampling dates ($p \leq .0034$).

Differences in growth were also noted between sites (Fig. 1). In

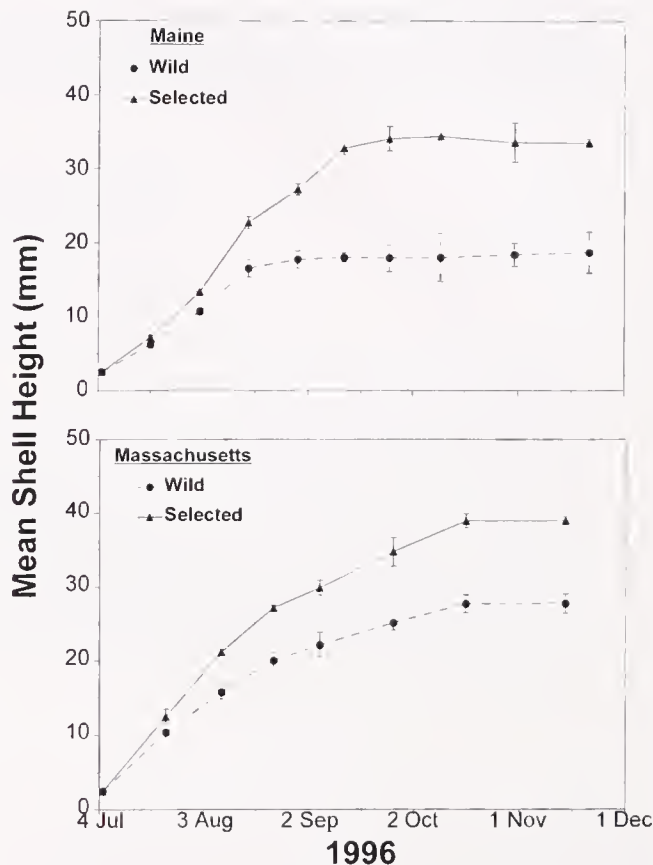


Figure 1. Mean shell height (mm, ± 1 SD) of selected and wild oysters, *C. virginica*, at North Bay, Massachusetts and Damariscotta River, Maine sites.

the Damariscotta River, growth of wild oysters ceased after 16 August whereas in North Bay, growth continued until 17 October. For selected oysters, shell height at both sites was statistically similar through September 26. Mean shell height of both selected and wild groups was, however, significantly greater at the North Bay site than the Damariscotta River site ($p \leq .0002$) at the end of the study (mid-November). The difference in final mean shell height between sites was 9.0 mm for wild oysters compared with 5.4 mm for selected oysters.

Juvenile Oyster Disease was highly prevalent in the Damariscotta River in 1996. Growth of wild oysters ceased after 16 August and mortality, with characteristic valve cupping and conchiolin deposits on inner valve surfaces, was initially observed on 30 August. Mortality in both groups occurred primarily during a 1-month period (Fig. 2). For the wild group, mortality was greatest between 30 August and 25 September. Most mortality in the selected group occurred later, between the 12 September and 25 September sampling dates. Growth in the selected group ceased at the same time. Selected oysters affected by JOD exhibited characteristics similar to those seen in wild oysters. Little mortality occurred in either group after 25 September. Final mean cumulative mortality on 21 November was 95.7 (± 2.5 SD) % in the wild group compared with 11.2 (± 5.0 SD) % in the select group; this difference was statistically significant ($p \leq .0001$).

In North Bay, JOD was not seen until late in the growing season, and then its impact was minimal. As a result, growth of both groups was continuous over the study period, and valve cup-

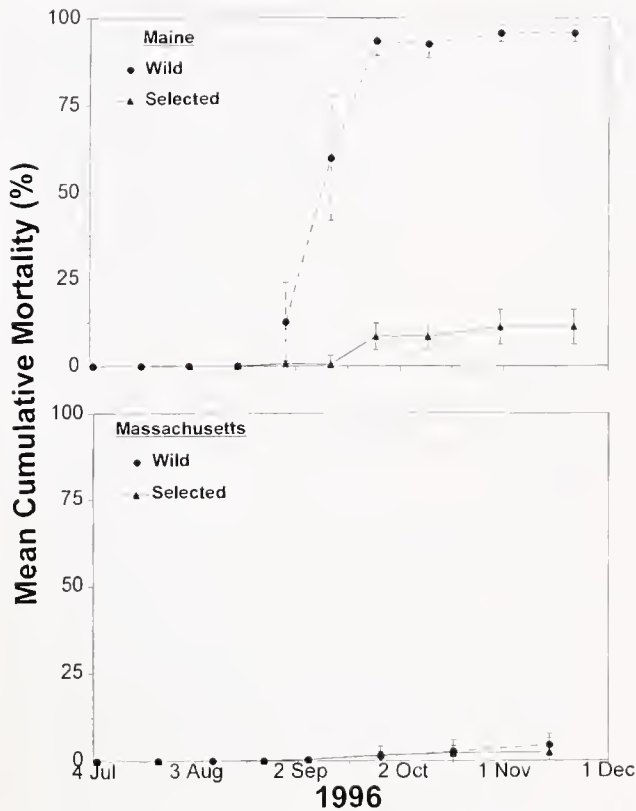


Figure 2. Mean cumulative mortality (%; ± 1 SD) of selected and wild oysters, *C. virginica*, at North Bay, Massachusetts and Damariscotta River, Maine sites.

ping and conchiolin deposits on inner valve surfaces were rare. Cumulative mortalities in both selected and wild groups were low (Fig. 2). Final mean cumulative mortality on 14 November was 2.5 (± 3.9 SD) % in the selected group and 4.6 (± 3.2 SD) % in the wild group; this difference was not statistically significant ($p = .2187$).

Differences in the impact of JOD between the two sites was further illustrated by differences in cumulative mortality (Fig. 2). Final mean cumulative mortality was significantly greater in the Damariscotta River than in North Bay for both selected and wild oysters ($p \leq .0079$).

Water temperature was generally greater in North Bay than in the Damariscotta River, particularly in July and August (Fig. 3). The maximum daily temperature recorded in North Bay was 25.5°C; in the Damariscotta River it was 24.7°C. Water temperature at both locations began to decline in September. Overall, mean daily temperature in North Bay (18.5°C) was significantly greater ($p \leq .0004$) than in the Damariscotta River (16.0°C).

Salinity at both sites was between 21 and 30 ppt throughout the entire study period (Fig. 3). In July, salinity was greater in North Bay than in the Damariscotta River, but from early August onward, salinity was greater in the Damariscotta River than in North Bay.

DISCUSSION

The fact that JOD did not manifest itself in North Bay at the same time and to the extent that it did in the Damariscotta River indicates that either the causative agent was not present at the time that seed was deployed or the environmental conditions were not

suitable for JOD development. Temperature (22–26°C) and salinity (22–30 ppt) in North Bay, however, were well within the range of those preferred by JOD (Bricelj et al. 1992, Lewis et al. 1996). If anything, given the generally greater water temperature in North Bay, it might be expected that the disease would have been evident sooner in North Bay than in the Damariscotta River, had the causative agent been present. Thus, it is likely that the causative agent of JOD was not initially present and therefore was not transported with the oysters from their production site in Maine to the commercial lease in Massachusetts. This supports previous findings that occurrences of JOD are site specific and cannot be related to a single hatchery or common broodstock (Bricelj et al. 1992).

The major differences in performance of oysters revealed in this study can be attributed to a combination of genetic and environmental factors. The selected group grew faster than the wild group at both locations. This supports the findings of previous studies reporting improved growth in bivalves as a result of selective breeding (Newkirk and Haley 1983, Davis et al. 1990, 1991, Hadley et al. 1991). The finding that the wild group grew much faster in North Bay than in the Damariscotta River probably had more to do with differences in JOD pressure than water temperature between the two sites, given that cessation of growth of wild oysters in the Damariscotta River occurred in conjunction with initial JOD mortality rather than a decrease in water temperature. Without the negative effects of JOD on growth in North Bay, wild oysters thus attained a greater size than in the Damariscotta River. The difference in growth of selected oysters between sites was about half that of wild oysters. Growth of selected oysters was similar at both sites until JOD in the Damariscotta River began causing mortality in this group. Further, growth of selected oysters

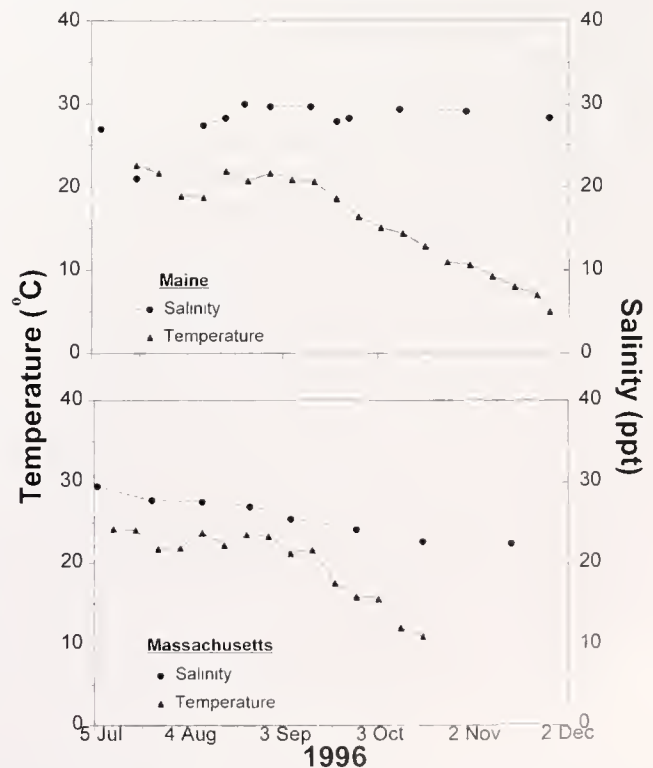


Figure 3. Weekly salinity (ppt) and weekly mean water temperature (°C) at North Bay, Massachusetts and Damariscotta River, Maine sites.

in the Damariscotta River continued for a longer period of time and mortality was delayed relative to wild oysters. This is evidence of an inherent tolerance of JOD which allowed selected oysters in the Damariscotta River to attain a closer size, in spite of heavy JOD, to those in North Bay, where prevailing temperature was greater and JOD was not a factor. Thus, differences in growth of selected oysters observed between sites were more likely a reflection of differences in JOD pressure than the 2.5°C daily temperature differential.

Mortality of both selected and wild oysters was minimal in North Bay because JOD did not manifest itself until late in the growing season, after which both groups had exceeded 25 mm in mean shell height. In the Damariscotta River, however, heavy challenge by JOD resulted in differential mortality between selected and wild groups. Greater survival of the selected group can be attributed to either faster growth or a greater disease resistance derived from previous selection by JOD, or a combination of both. Previous research has demonstrated that once oysters attain a shell height of 25–30 mm, mortality caused by JOD is 20% or less (Bricelj et al. 1992, Davis and Barber 1994). More important than size at the time of mortality, however, is size at the time of initial exposure to JOD infectious agent(s). Barber et al. (1996), in a study of the seasonal impacts of JOD, found that a cohort having a mean shell height of 27.1 mm at the end of July, 3–4 weeks prior to the onset of major mortality in smaller cohorts, had a final cumulative mortality of 19%; cohorts having a mean shell height of 22 mm or less experienced mortality ranging from 65 to 95%. In all cases, signs of JOD did not appear until 3–4 weeks after initial deployment; it was concluded that this period of time represented an incubation period for the JOD etiological agent (Barber et al. 1996). In this study, mortality of wild oysters in the Damariscotta River began in late August. Three weeks before the onset of mortality (2 August) thus represents the time of first exposure to JOD etiological agent(s). On this date, mean shell height of the selected group was 13.2 mm and mean shell height of the wild group was 10.6 mm, both well below the 25 mm refuge size. Thus, on the basis of size alone, it would be expected that both groups of oysters would have experienced similar mortality.

The fact that cumulative mortality in the selected group was only 11.2% (compared with 95.7% in the wild group), however, suggests strongly that this group of oysters has a genetically based tolerance of JOD as a result of previous exposure to and selection by the disease. Indeed, the selected line used in this study (Flowers F₃) is the offspring of previous generations that have been grown in the Damariscotta River, where JOD has been endemic since 1988 (Davis and Barber 1994, Barber et al. 1996, Davis and Barber unpublished data). Farley et al. (1997, 1998) used survivors of JOD outbreaks exhibiting shell checks (presumably due to the effects of JOD) in New York to produce progeny that exhibited survival rates 2.5–35 times greater than those of susceptible oysters of the same age, depending on location. Thus, it appears that local environments can influence the performance of a selected line at a particular location, as was noted by Rawson and Hilbish (1991) for growth in juvenile *Mercenaria mercenaria*.

Genetic selection of oysters for disease resistance has been previously documented. In cases where diseases have caused mas-

sive mortality in host populations, natural selection has resulted in increased disease tolerance in offspring of surviving organisms. This has been reported for populations of *C. virginica* affected by Malpeque disease and diseases caused by protozoan parasites *Perkinsus marinus*, *Haplosporidium costale*, and *H. nelsoni* (Needler and Logie 1947, Andrews and Hewatt 1957, Andrews and Castagna 1978, Haskin and Ford 1979). Evidence of naturally acquired disease resistance to *H. nelsoni* mortality in populations of *C. virginica* in Delaware Bay led to a program of selective breeding to ascertain whether improved survival was heritable and could be enhanced relative to natural populations (Ford 1988). Ford and Haskin (1987) reported improved survival of oysters through six generations of selection by *H. nelsoni*.

In spite of success at selecting for disease resistance, little is known of the defense mechanisms that lead to improved survival in selected strains. The absence of a clear defense mechanism has led to the conclusion that resistance to *H. nelsoni* mortality is under the control of multiple genes, and results in selected oysters being generally superior physiologically (Ford and Haskin 1987, Barber et al. 1991). Similarly, Baud et al. (1997) found that strains of oysters, *Ostrea edulis*, selected for resistance to the parasitic protozoan, *Bonamia ostreae*, grew faster and had lower mortality than control groups in the absence of disease pressure, but under less than optimal nutritional conditions. It was suggested that in this case, selection for disease resistance had resulted in an increased tolerance of stress in general. Identification of the mechanism of resistance to JOD mortality awaits determination of an etiological agent.

The results of this study provide another approach for improving oyster production in areas where JOD is endemic. As previously reported, the effects of JOD can be minimized by obtaining seed that is greater than 25 mm in shell height at the time of exposure to JOD (Davis and Barber 1994, Barber et al. 1996). This size can be achieved by conditioning broodstock out of season so as to obtain seed early (May) or late (August) in the year. Producing seed early in the year will ensure that a size >25 mm is attained before the onset of JOD. Placing seed in nursery trays after mid-August will avoid exposure to JOD that summer but requires holding the seed over the winter; subsequent deployment early the following spring will ensure that size-based tolerance to JOD is attained. Continued selection for fast growth will decrease the time required for oysters to attain the critical size refuge. The finding reported here, that oysters selected for fast growth in the Damariscotta River, Maine, also have a genetically based resistance to JOD mortality, will serve to further reduce the impacts of this disease.

ACKNOWLEDGMENTS

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SYSTEMIC GREGARINE-LIKE PROTOZOA IN JUVENILE PACIFIC OYSTERS, *CRASSOSTREA GIGAS* (THUNBERG 1793)

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ABSTRACT During a detailed survey of Pacific oyster, *Crassostrea gigas*, juvenile health at intensive rearing facilities during 1996 and 1997, systemic Gregarine-like protozoa were found in seed oysters planted on certain nursery beds. The organisms were never found in seed oysters from intensive production tank facilities. The infections were not associated with morbidity and mortality and appeared to be of limited pathologic significance. The organisms occurred systemically in seed oysters and were most commonly found in vesicular connective tissue with minimal to no associated host response. Oyster seed planted on three nursery bed areas in Washington became infected but those planted in Arcata Bay, California never became infected. Infection intensity and prevalence initially increased after planting, then declined. The organisms are extremely rare in adult oysters and the data suggest that juvenile oysters essentially outgrow the infections without detectable clinical pathologic effects. Spore stages were never observed and a definitive taxonomic affinity could not be established.

KEY WORDS: Bivalve mollusc, Gregarine-like, health management, aquaculture

INTRODUCTION

Production of bivalve molluscs, including the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), is practiced in Washington and other west coast states. Since the late 1970s this shellfish industry has become increasingly based on intensive hatchery and nursery production of larval and seed shellfish. It has also pioneered the development of many new features of intensive hatchery and nursery production for various species of bivalves. These seed and larval products are disseminated along the Pacific coast of North America and to other regions.

To increase the efficiency of juvenile Pacific oyster production and to initiate a comprehensive health management program, including ongoing surveillance for infectious diseases, we began an oyster seed health program in 1996 in California, Hawaii, Oregon, and Washington. This program intentionally focused on the gross and microscopic examination of organs and tissues of shellfish to provide an assessment of lesions and disease processes. One of our objectives in using this approach was to avoid the mistake of microbiologically characterizing real or apparent shellfish diseases without understanding the nature, course, and consequences of the disease on the affected bivalves.

During the shellfish seed health survey, we discovered the presence of apparently benign systemic protozoa in early stage juvenile oysters planted on certain nursery beds. Similar organisms have been briefly reported previously in adult manila clams (*Tapes philippinarum*, Adams and Reeve, 1850) from British Columbia (Bower et al. 1992) and referred to as Gregarine-like Apicomplexa, although their taxonomic affinity has not been definitively established. Similar individual organisms have also been rarely observed by the senior author of this paper and others (personal

communications, S. Bower, T. Meyers) since about 1982 in the tissues of adult Pacific oysters. In adult oysters and manila clams, these organisms are considered to have limited or no pathologic significance. During the course of this study we discovered a higher intensity and prevalence of these Gregarine-like organisms in juvenile oysters planted under certain conditions. Although the organisms also appear to be of limited pathologic significance in juvenile oysters, it is important to document their occurrence, significance, and key features regarding conditions under which the oysters can become infected.

MATERIALS AND METHODS

Over 200 cultures of juvenile Pacific oysters, *Crassostrea gigas*, were examined sequentially through their development during 1996 and 1997. The shellfish were collected both within intensive commercial production facilities from tanks and upweller systems and on oyster seed beds in Washington and California. Seed oysters originating from intensive nurseries were sampled from the tank facilities prior to being planted on nursery beds. The water filtration systems at each nursery facility varied, but at a minimum, consisted of sand filtration providing a nominal 50 μ m filtration. Juvenile oysters placed on beds were seed set on cultch and placed in stacked vexar bags. These bags were then placed on bottom grounds in traditional nursery areas in North Puget Sound, Grays Harbor, and Willapa Bay, Washington and in Arcata Bay, California. Three batches of seed oysters were used in the study and planted at an average shell height of between 1 and 5 mm in August and November 1996 and May 1997 (Tables 1 and 2) on three nursery beds in Washington and one in California.

TABLE 1.
Infection by Gregarine-like organisms of hatchery seed placed on nursery beds in August 1996.

Location	Sample Date	Number Examined	Avg Shell Height (mm)	Prevalence of Infection (%)	Mean Infection Intensity ^a
Hatchery stock	7/31/96	62	4.5	0	
N. Puget Sound, WA	9/25/96	14	11.6	43	8.6
	10/22/96	170	9.2	41	3.7
	11/26/96	22	9.2	68	16.6
	1/8/97	24	9.4	92	8.5
	2/10/97	25	10.4	96	9.0
	4/8/97	30	16.3	100	20.3
	5/27/97	36	10.1	100	11.5
	7/8/97	34	20.3	85	4.8
Grays Harbor, WA	10/30/96	22	15.9	55	4.6
	1/9/97	13	19.8	62	6.8
	4/8/97	24	26.7	38	2.3
Willapa Bay, WA	10/29/96	24	8.3	96	15.8
	1/9/97	16	8.4	94	10.0
	7/9/97	17	16.6	88	6.5
Arcata Bay, CA	12/2/96	79	9.7	0	
	1/9/97	58	14.8	0	
	3/12/97	46	23.2	0	

^a See Materials and Methods for calculation method of mean infection intensity.

During the study, juvenile oysters were transported to the laboratory chilled where they were examined within 24 h of sampling. Shell height of up to 30 randomly chosen oysters was measured and representative oysters were preserved for histological examination. Oysters less than about 8 mm shell height were fixed whole in the shell in Davidson's fixative and further decalcified as needed. Oysters less than 5 mm in shell height were embedded in a plastic histological medium (Elston et al. 1982). Larger oysters were prepared for histological examination using routine paraffin embedding techniques. Sections were routinely stained with hematoxylin and eosin and selected specimens were stained with Giemsa, periodic acid-Schiff (PAS), and an acid-fast procedure (Luna 1968).

During histological examination, our target was to evaluate at least 30 individuals from each sample, providing a 95% confidence level of a presumed detection sensitivity of 10% (Amos 1985). In making histological examinations to enumerate the Gregarine-like protozoa, we eliminated from consideration all those

histological sections that did not show a complete transverse section of the body mass from the dorsal aspect (hinge ligament regions) to the adductor muscle, as well as associated tissues. This method enhanced the uniformity of examination but, in some cases, resulted in a reduction of individuals examined in particular samples.

Prevalence of the Gregarine-like organisms was recorded and intensity of infection was estimated based on the following system: infection intensity category 1, 1 to 10 organisms per individual; infection intensity category 2, 11 to 20 organisms per individual; infection intensity category 3, 21 or more organisms per individual. Rarely were more than 50 organisms found per individual. In order to create an infection intensity index, the median number of organisms per category was assigned to each infected individual (6 for category 1, 16 for category 2, and 36 for category 3) and summed for the given sample. This number was divided by the number of individual oysters in the sample to provide the infection index for each sample.

TABLE 2.
Infection by Gregarine-like organisms of hatchery seed planted on seed nursery beds in November 1996 and May 1997.

Location	Sample Date	Number Examined	Average Shell Height (mm)	Prevalence of Infection (%)	Mean Infection Intensity ^a
November 1996					
Hatchery stock	11/26/96	30	1.5	0	
N. Puget Sound	1/8/97	111	2.6	0	
	4/8/97	30	3.7	7.0	0.5
	7/8/97	30	15.0	50.0	3.0
May 1997					
Hatchery stock	5/2/97	93	1.5	0	
N. Puget Sound	7/8/97	24	6.2	4.1	0.3

^a See Materials and Methods for calculation method of mean infection intensity.

To estimate the prevalence of the Gregarine-like protozoa in additional intensively reared nursery seed and in field-planted adult oysters, we examined records of examinations of 2,580 seed oysters and 630 adult oysters from Washington state made by the senior author of this paper since 1995.

RESULTS

Mortality Estimation and Clinical Observations

Samples of juvenile shellfish used for this study and examined during the 1996–1997 seed health surveillance program are listed in Tables 1 and 2. None of the samples from which we found the Gregarine-like Apicomplexa were characterized by any clinically observable signs of morbidity or any mortality episodes.

Histological Characterization of Organisms and Host Response

The Gregarine-like organisms were found within the circulatory system in various tissues but most commonly in the vascular spaces of the vesicular connective tissue around the digestive organs. Occasionally, they were observed in the vasculature of the

gill. They were found at any location within the vasculature of the connective tissue but often were concentrated near the intestine and particularly at the posterior aspect of the body near this organ. Intensity of infection was variable, as shown below, and can be visualized from a low to medium to high intensity in Figure 1a to 1c, respectively, which corresponds to infection intensity categories 1 to 3. In the high infection intensities, the organisms tended to accumulate in focal concentrations (Fig. 1c).

The Gregarine-like organisms were ovoid to egg-shaped and measured up to 17.6 μm in length by 10.5 μm in width and contained a central nucleus with prominent dark staining nucleolus (Fig. 1d). They were nonreactive to PAS stain, and Giemsa stain did not differentiate any structures not visible in H&E stained sections. The organisms were bounded by a simple membrane and did not display any structures representing spore walls or spore formation.

Minimal host inflammatory response was associated with the occurrence of these organisms. There was no clear or marked increase in cellularity, as would be expected from hemocyte infiltration, even in the heaviest infections. Occasionally, Gregarine-like organisms were found surrounded by single host hemocytes

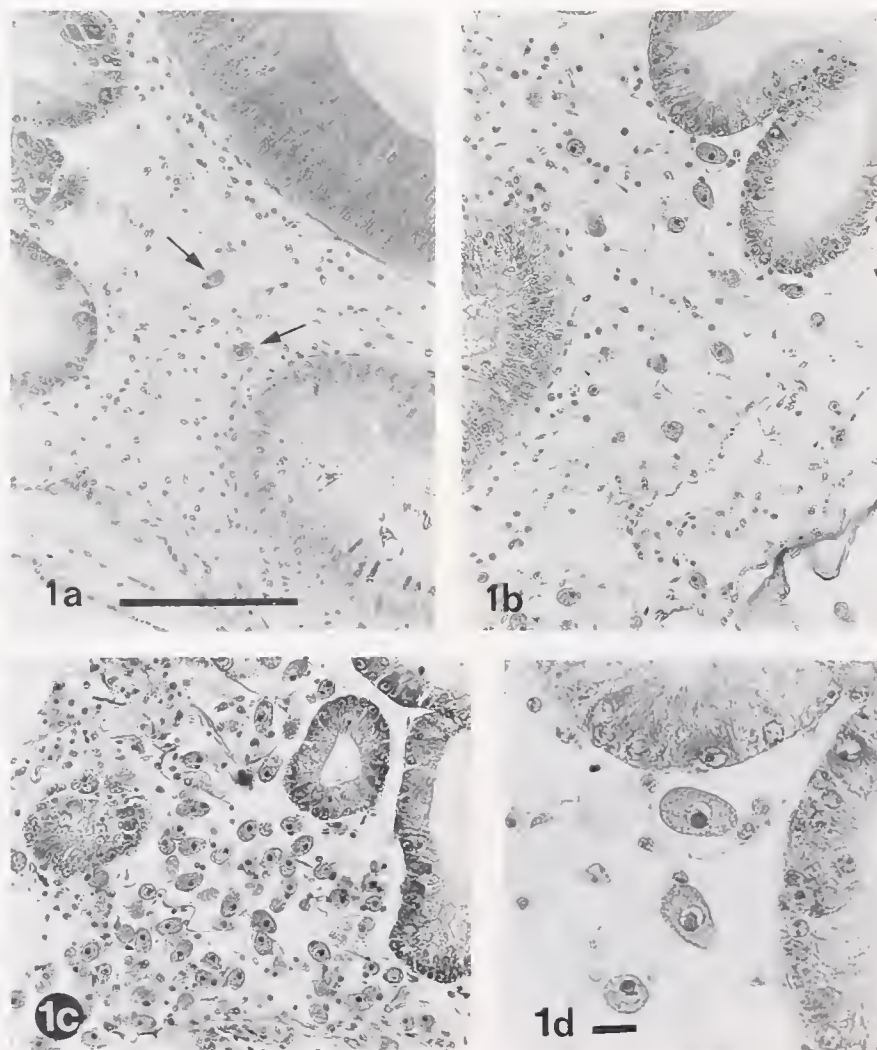


Figure 1. Gregarine-like organisms in the interstitial vascular spaces of juvenile Pacific oyster connective tissue. (a) Infection intensity category 1 with two organisms shown at arrows; (b) infection intensity category 2; (c) infection intensity category 3 showing a focal accumulation of the organisms; (d) detailed view of three organisms. Bar = 100 μm in a, b and c; bar = 10 μm in d; all hematoxylin and eosin.

(Fig. 2) indicating some degree of recognition of the organisms by the oysters as nonself material.

Prevalence, Intensity, and Distribution of Infections

The Gregarine-like organisms were never found in the three samples from the 1996 to 1997 study totaling 185 juvenile oysters held within intensive tank rearing facilities in Washington (Tables 1 and 2). A review of seed examination records conducted from the same facilities since 1995 found no occurrences of the organisms in an additional 2,580 seed oysters examined.

During the seed health surveillance study, however, oysters planted on three bed areas in Washington were infected at a prevalence of up to 100% with varying intensities (Tables 1 and 2). Seed planted in August 1996 in North Puget Sound became the most heavily infected, increasing in prevalence to 100% by April 1997 but then declining slightly in prevalence by July 1997. The infection intensity index was more variable but followed a similar pattern, reaching a peak in April 1997 but declining markedly by July 1997, as the oysters increased in size. A somewhat similar pattern of infection was noted in seed planted in Grays Harbor and Willapa Bay, Washington, but both prevalence and infection intensity were lower. No infected seed were found in a total of 183 seed oysters planted and examined from the Arcata Bay, California site.

Seed planted in November 1996 and May 1997 also became infected but at a lower prevalence and intensity than seed planted in August 1996. A maximum prevalence of 50% was found and a maximum infection intensity of 8.6 was recorded, in comparison with a maximum infection intensity of 20.3 in the seed planted in August 1996.

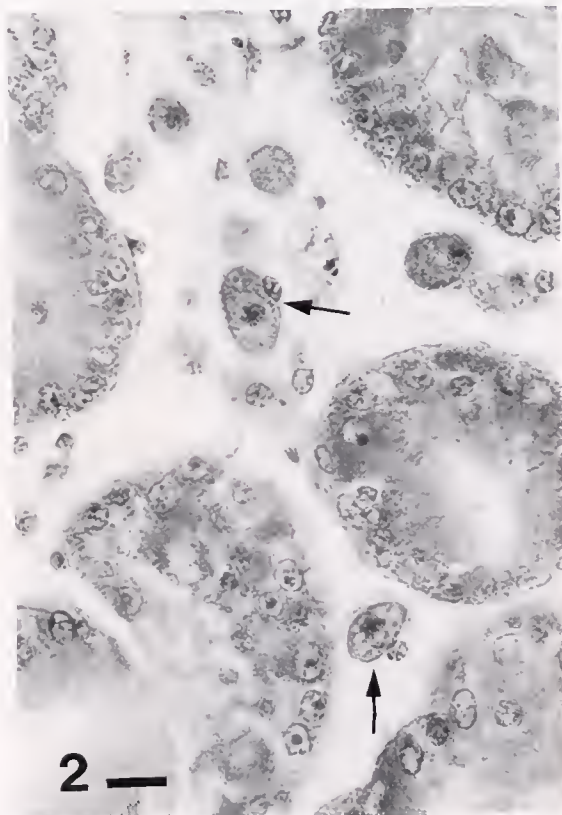


Figure 2. Gregarine-like organisms in juvenile Pacific oysters. Two organisms at arrows have been surrounded by single host hemocytes. Bar = 10 μ m; hematoxylin and eosin.

In the review of examination records since 1995 from Washington oyster beds, no organisms were found in 630 adult oysters.

DISCUSSION

Occurrence and Pathologic Significance

These systemic protozoa appear to have limited pathologic significance for Pacific oysters. Even in the heaviest infections observed, there was no tissue damage observed by histology and only limited suggestions of a host inflammatory response. At the heaviest infections, the organisms might consume significant nutritional resources from the seed oysters but no clinical effects were evident.

The oyster seed appear to essentially outgrow the infection as they increase in size above 2 cm shell height. We observed a small decrease in infection prevalence and a decrease in infection intensity in all of the July 1997 samples of seed planted on nursery beds in August 1996. The infection intensity of the oysters planted in November 1996 actually increased between April and July 1997 but was still lower than comparable prevalence and intensity data from the seed planted in August 1996, and the seed was smaller than that of the former group. The seed planted in May 1997 showed only a relatively low prevalence and infection intensity by July.

The extremely low prevalence in adult Pacific oysters, which was too low to be numerically estimated from a sample of the 630 adult oysters examined but is known from rare but unquantified previous anecdotal reports, demonstrates that at some stage of development the infections are either eliminated or diluted by growth of tissue to the point where they become undetectable. It is doubtful that the infection disappears from the adult oyster population because of the death of infected seed because there is no indication of tissue damage, morbidity, or mortality in the seed oysters. Although we did not follow the infections in oysters beyond a shell height of 2 to 3 cm in this study, the declining infection intensity and prevalence in the three samples of largest seed, which was planted in August 1996, supports the view that oysters outgrow the infection.

These observations suggest that both time of planting and seed size are related to the infection prevalence and intensity in seed oysters. Data from the three planting dates showed that the highest infection intensities occurred from November to May in the seed planted in August 1996. The infection intensities of seed actually planted in November and May never reached levels comparable to those reached by the seed planted in August. This suggests that the source of infection may be present over the entire year but at a higher level during the summer and fall months.

Similarly, in Manila clams, as reported by Bower et al. (1992), the infection of Gregarine-like Apicomplexa that resemble those in the oysters are reported to be of limited or no pathologic significance, although it is not known if the clam and oyster organisms represent the same species. However, both species may represent an aberrant host for their respective organisms. Thus, it is possible that these organisms have a well-developed host-parasite relationship in some other species. Gregarine-like organisms are reported from the digestive tract of penaeid shrimp (Lightner 1996) but are also considered to be of limited pathologic significance unless they occur at very high infection intensities.

It is interesting to note that whatever the normal host of this species is, it appears to be present in several embayments of Washington state but not in Arcata Bay, California. It is also important to note that the organisms are not present in seed held in intensive

nursery systems which received sand-filtered water. Therefore, it is reasonable to conclude that there is essentially no chance of disseminating the organisms by the geographic distribution of seed from these facilities.

Identity of the Organisms and Significance of Gregarines

There was no indication of spore formation in these organisms and therefore no information on which to base a taxonomic identification. As noted above, Bower et al. (1992) referred to morphologically similar organisms in adult manila clams as Gregarine-like Apicomplexa. It should be emphasized that the taxonomic identity of the organisms we report on here is unknown, and morphologic similarities to Gregarines may be superficial and of no taxonomic significance.

In the manila clams, Bower et al. (1992) described pear-shaped organisms, similar in size to those reported here, occurring extracellularly between epithelial cells of the gastrointestinal tract and also showed a focal infection in the circular muscle of the digestive tract. These infections caused some distortion of the gut but minimal inflammatory response. In adult manila clams from Redonda Island, the authors found 8/10 clams infected and most were considered light infections. They did not find dividing forms; all were associated with the gut and found extracellularly, and appear to be approximately the same size as the organisms described in this paper.

Gregarine organisms have been described from Eastern oysters, *Crassostrea virginica* (Gmelin 1791). Early reports ascribed morbidity and mortality to infection by *Nematopsis ostrearum* (Prytherch 1940) and *Nematopsis prytherchi* (Sprague 1949). In a subsequent series of experimental studies, Sprague and Orr (1955) could not confirm or refute the Prytherch (1940) view that *Nem-*

atopsis is a significant pathogen. These authors noted that cephaline gregarines are not known for being highly detrimental to their hosts, that these species do not reproduce asexually, and that numbers of spores do not increase in the host by reproduction of the parasite in the host. The organisms are generally considered to have limited pathologic significance. Alternatively, Sawyer et al. (1975) reported a gregarine-like parasite from *Crassostrea virginica* that was associated with focal sloughing of digestive tract epithelium.

Nascimento et al. (1986) reported *Nematopsis* in *Crassostrea rhizophorae* from Brazil but reported no unusual mortality of oysters that could be attributed to the infection; they also stated that there was no evidence of any harmful effect of the parasite on the oysters. They showed parasite cysts in interstitial spaces between the connective tissue fibers with no inflammatory reaction and concluded that the parasite is a resting stage which does not propagate or grow within the oyster.

This study documents organisms of unknown taxonomic affinity but with at least superficial resemblance to Gregarine-like Apicomplexa in juvenile Pacific oysters and demonstrates their limited pathologic significance, as well as their absence from tank-reared seed. Hopefully, it will also help prevent future misidentification of these organisms as more significant molluscan pathogens, such as haplosporidians, which may have previously occurred.

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CONDITIONING OF EASTERN OYSTERS IN A CLOSED, RECIRCULATING SYSTEM

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ABSTRACT Techniques were developed for holding and conditioning of eastern oysters, *Crassostrea virginica* (Gmelin), in a recirculating system. Oysters collected in February from public oyster grounds off the coast of Louisiana were maintained in a recirculating system for 8 wks. For conditioning, water temperature in the system was gradually raised with a heat pump from 14°C, and held at 25°C for 6 wks. Oysters were fed a diet of algal paste (*Isochrysis galbana* for the first 6 wks and *Chaetoceros calcitrans* for the last 2 wks). Water quality, mortality, *Perkinsus marinus* infection, gonad development, and physiologic condition (dry tissue-to-dry shell ratio, dry tissue-to-wet tissue ratio, digestive diverticula tubule ratio) were monitored. At weeks 7 and 8, the laboratory-held oysters were compared with field controls held at Grand Isle, Louisiana. Water quality in the system remained within target ranges. Mortality was low (18 of 300 oysters stocked) and not associated with *P. marinus* infection. In the laboratory at week 1, the gonads of all oysters sampled were classified as immature or in early development. By week 5, the gonads of 73% of oysters sampled were classified as mature. Physiologic condition decreased in the laboratory. Field controls reached a higher mean gametic stage and were in better physiologic condition at the end of the 8-wk study. These differences were attributed to differences in nutrition available between the field and laboratory. This study demonstrated that conditioning of *Crassostrea virginica* is possible in a closed, recirculating system, although improvements in nutrition would be useful.

KEY WORDS: *Crassostrea virginica*, conditioning, gametogenesis, histology, aquaculture, recirculation

INTRODUCTION

The eastern oyster, *Crassostrea virginica* (Gmelin 1791), supports a valuable commercial fishery. The annual harvest value is measured in millions of dollars in the United States (MacKenzie 1996). However, the industry has been plagued with multiple problems in recent years, resulting in decreased oyster production (MacKenzie 1996, Andrews 1991) and creating a need for research in oyster genetics.

This has made necessary the development and improvement of culture techniques, including design of recirculating systems for holding and conditioning of broodstock. Typically, work is limited to the natural spawning period (April to October), and even then oysters require repeated monitoring for gonadal maturation. Additionally, research away from the coast requires continual monitoring and transport of oysters for use in the laboratory. Further problems include the costs and labor associated with obtaining suitable water sources. Thus, development of a recirculating system for holding and ripening of oysters in the laboratory would extend the oyster spawning season and expand research opportunities. In addition, such systems would allow containment of genetically modified organisms, including those produced by gene transfer (Kapuscinski and Hallerman 1991). The ability to condition oysters in an artificial system is a first step toward containment of the complete life cycle in the laboratory.

The reproductive ecology of *C. virginica* is well described (Shumway 1996, Thompson et al. 1996). The primary cue for gamete development seems to be temperature, and research has addressed the effects of temperature on gametogenesis (Price and

Maurer 1971, Loosanoff and Davis 1953). Gametogenesis and spawning begin with increasing temperatures in the spring and summer, and the existence of oysters acclimatized to local environments, with specific temperature requirements in reproduction, have been reported (Loosanoff 1969). Oysters are routinely conditioned through temperature manipulation in research hatcheries using natural seawater and foods (Dupuy et al. 1977). Laboratory studies on temperature and gametogenesis have utilized natural seawater for water exchange in holding systems (Robinson 1992, Price and Maurer 1971). Some research has been conducted on the maintenance of oysters in a laboratory environment. Tolerance levels of oysters to various water quality conditions have been reported (Epifanio and Srna 1975, Epifanio et al. 1975), as have guidelines for feeding regimes and rations (Epifanio and Ewart 1977). Several studies have also addressed design and maintenance of recirculating systems (MacMillian et al. 1994, Thiekler 1981).

The goal of this project was to develop techniques for holding and conditioning of *C. virginica* in a recirculating system. To our knowledge, this is the first study on the conditioning of oysters in such a system. Oysters collected during winter in Louisiana coastal waters were brought into the laboratory, and conditioning was attempted over an 8-wk period by incremental raising of temperature from 14°C to 25°C. Laboratory-held oysters were compared with oysters from natural waters before and after the holding period. Our objectives during the study were to monitor (1) water quality, (2) mortality and disease, (3) changes in gonad condition, and (4) changes in physiological condition.

MATERIALS AND METHODS

Eastern oysters were collected from public oyster grounds in Hackberry Bay Louisiana (29°40'00"N, 90°02'30"W) on February 19, 1997. Hackberry Bay oysters were selected because of reportedly low levels of *Perkinsus marinus* infection in this population (Supan, unpublished data). The oysters were kept on ice, and transferred to the Louisiana State University Aquaculture Research Station (ARS) on February 21. Of these, 50 were processed upon arrival at the ARS to establish a baseline reference. Another 600 oysters were cleaned of external debris and split into two groups of 300 each.

One group was transported to the Louisiana State University oyster hatchery at Grand Isle, Louisiana (29°12'30"N, 90°02'30"W) to serve as a field reference. These were suspended from a pier in 1.25-cm mesh ADPI® bags, and were exposed to ambient conditions over the course of the experiment.

The second group was placed in a closed, recirculating system in the laboratory for conditioning (Fig. 1). The system was composed of two 500-L rectangular, fiberglass tanks (259 cm × 91 cm × 20 cm) connected to a 1500-L sump (244 cm × 122 cm × 65 cm). Standard PVC pipe (5.08-cm, schedule 40 pipe, unless otherwise indicated) was used for all plumbing. Each tank had a ball valve to control water delivery, a Venturi orifice on the water inlet for aeration, and an internal standpipe to control water level. Temperature was maintained within 1°C by an in-line heat pump (1-hp, 13,300 BTU/hr; ACRY-TEC, San Diego, CA) with an electric remote control (model T775B, Honeywell Inc., Golden Valley, MN) between the sump and holding tanks. A separate loop from

the sump provided filtration and sterilization. In this loop, water flowed through a 0.30-m³ upwelling bead filter (Malone et al. 1993) and a 25-W ultraviolet light (Rainbow Plastics, El Monte, CA). Nitrifying bacteria in the effluent of a functioning bead filter were used to inoculate this filter. Bacterial growth in the filter was promoted with the addition of sodium nitrite and ammonium chloride for 4 wks before the study began (Malone and Manthe 1985). Two 5.2-cm diameter PVC foam fractionators were constructed (Wheaton 1977) and used in an additional sump loop to remove dissolved organics when excessive foaming was noticed. A ¾-hp centrifugal pump (Maxim, Moorpark, CA) was attached to the sump and drove water through the system. The system was filled with artificial salt water (Fritz Super Salt, Fritz Industries Inc., Dallas, TX) at a salinity of 15 ppt.

The laboratory oysters were fed a diet of algal paste produced from a continuous turbidostat culture maintained at the ARS (Theegala 1997). Paste was stored refrigerated until use (within 2 months). *Isochrysis galbana* (clone T-Iso) was fed for the first 6 wks and *Chaetocerus calcitrans* for the final 2 wks. The amount of paste fed was calculated based on dry weight (Epifanio and Ewart 1977), and prepared daily in the following manner: 140 g of paste was removed from storage at 4°C, weighed, dissolved in system water, and passed through 75-µm and 15-µm nylon mesh to disperse the algal cells. This mixture was added to the oyster tanks and allowed to circulate through the system. The backwash effluent from the beadfilter was returned to the sump daily, allowing total conservation of salt water, and providing a potential nutritional supplement of bacteria and dissolved organics to the oysters.

The study lasted 8 wks, from February 21 to April 15, 1997. Oysters were acclimated during week 1 in the system at 15°C. At day 10, temperature was increased 2°C every 2 days until reaching 25°C, which was maintained for the remainder of the study. Laboratory samples of 50 oysters were removed from the tanks at weeks 1, 3, 5, 7, and 8 for analysis of disease, gonad development, and physiologic condition. Of each sample, 30 oysters were assayed for *P. marinus* infection and sectioned for histology. The remaining 20 were processed for determination of physiologic condition. On weeks 7 and 8, 50 reference oysters were brought from Grand Isle as field samples and processed as described above. The whole wet weight of every oyster processed was recorded.

Water Quality

Water quality was monitored weekly (although daily measurements were made during weeks 2 and 3). A Hach® test kit (Model FF-3, Loveland, CO, USA) was used to measure ammonia con-

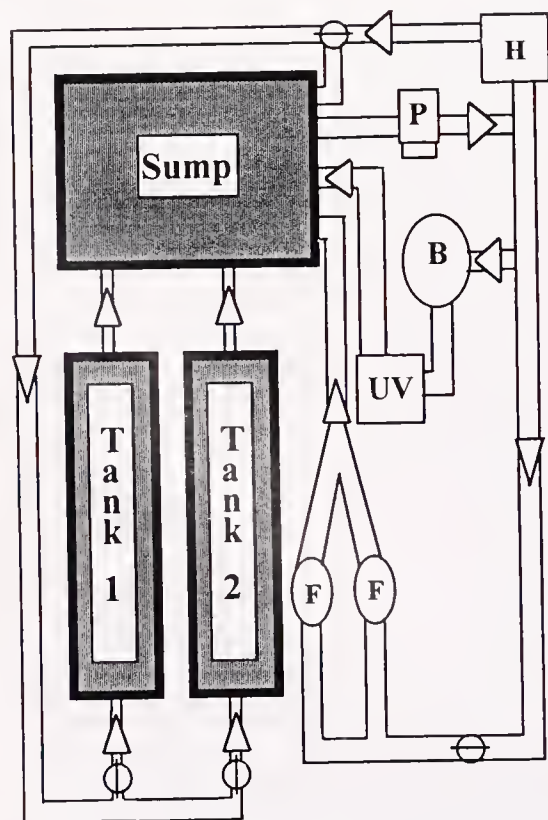


Figure 1. Design of recirculating system used to condition *Crassostrea virginica*. B = biological filter, H = heat pump, P = pump, F = foam fractionators, UV = ultraviolet light.

TABLE 1.

Comparison of observed and target water quality values in the recirculating system during an 8-wk study on conditioning of *Crassostrea virginica*

Parameter	Observed Values	Target Values
Salinity (ppt)	14.0–17.0	15.0
Temperature (°C)	13.0–26.0	15.0–25.0
Ammonia (mg/L)	0.10–4.2	0.0–5.5
Nitrite (mg/L)	0.05–1.90	0.0–460.0
pH	7.8–8.5	8.0–8.5
Dissolved oxygen (mg/L)	5.6–7.8	>3.6

TABLE 2.

Gametic stages assigned to histologic sections, and used to classify gonad development in *Crassostrea virginica*

Stage	Description
1 (immature)	Follicles small and contracted. Some early sex cells (such as pro-oogonia) may be poorly visible.
2 (developing)	<5% of all gametes mature.
3 (early maturity)	6–50% of all gametes mature.
4 (maturity)	51–75% of all gametes mature.
5 (late maturity)	76–100% of all gametes mature.
6 (regression)	Proliferation of hemocytes and cytolysis evident, some mature cells may remain.

centration (mg/L $\text{NH}_3\text{-N}$), nitrite concentration (mg/L $\text{NO}_2\text{-N}$), pH, hardness (mg/L CaCO_3), and alkalinity (mg/L CaCO_3). Dissolved oxygen was measured with a YSI oxygen meter (Yellow Spring, CO), and salinity with a hand-held refractometer. Tolerance levels from the literature (Galstoff 1964, Epifanio and Srna 1975, Epifanio et al. 1975) were used as guidelines for acceptable water quality conditions (Table 1). Temperature was measured daily with a submersible thermometer.

Mortality and Disease

Levels of *P. marinus* infection were measured using the fluid thioglycolate method (Ray 1966). A sample of rectal tissue was obtained aseptically and incubated in thioglycolate media for at least 7 days. The tissue sample was smeared on a glass microscope slide, stained with Lugol's iodine, and examined at 100 \times with brightfield microscopy for presence of hyphospores. Infection levels were assigned a value ranging from 0 (no detectable infection) to 6 (heavily infected, more than 1,000 hyphospores in a 5-mm field) (Quick and Makin 1971). From these values, infection prevalence (percent infected oysters per sample), intensity (average intensity of infection among infected oysters), and weighted

incidence (average intensity of infection for the entire sample) were calculated (Quick and Makin 1971).

Dead oysters (individuals with gaping shells) were identified and removed from the tanks daily. If possible, a small rectal tissue sample was collected and inspected for *P. marinus* infection (12 of 18).

Gonad Development

Gonad development was assayed histologically. Standardization of sectioning is necessary for histologic comparisons among oysters (Morales-Alamo and Mann 1989). Accordingly, processing involved removing a 4–5 mm cross-section of each oyster just posterior to the junction of the labial palp and gill, and preserving this tissue in Davidson's fixative (Howard and Smith 1983). A 4- μm section was obtained ~1 mm from the junction, mounted and stained with Gill's hematoxylin and eosin (Howard and Smith 1983). Sections were characterized using features identified by Morales-Alamo and Mann (1989). Gonad development was classified as one of six stages (Table 2). For each oyster sectioned, gametic stage and sex (male, female, hermaphrodite, or unidentifiable) were recorded. Observations were made with brightfield microscopy at 100 \times . The mean gametic stage for each sample was calculated.

Physiological Condition

Physiological condition was closely monitored in this study for several reasons. A primary concern was the usefulness of the algal paste as a complete food source for oysters. Other concerns were the effect of temperature stress, water quality, and *P. marinus* infection. Therefore, physiologic condition was characterized with three assays, two traditional condition indices and an assay based on the morphology of the digestive diverticula.

The two traditional condition indices used were the ratio of dry tissue-to-dry shell (Rainer and Mann 1992), and the ratio of dry tissue-to-wet tissue (Lucas and Benniger 1985). Oysters were cleaned of external organisms and debris, opened, and the tissues and shell were separated and weighed (wet weight). Tissues and

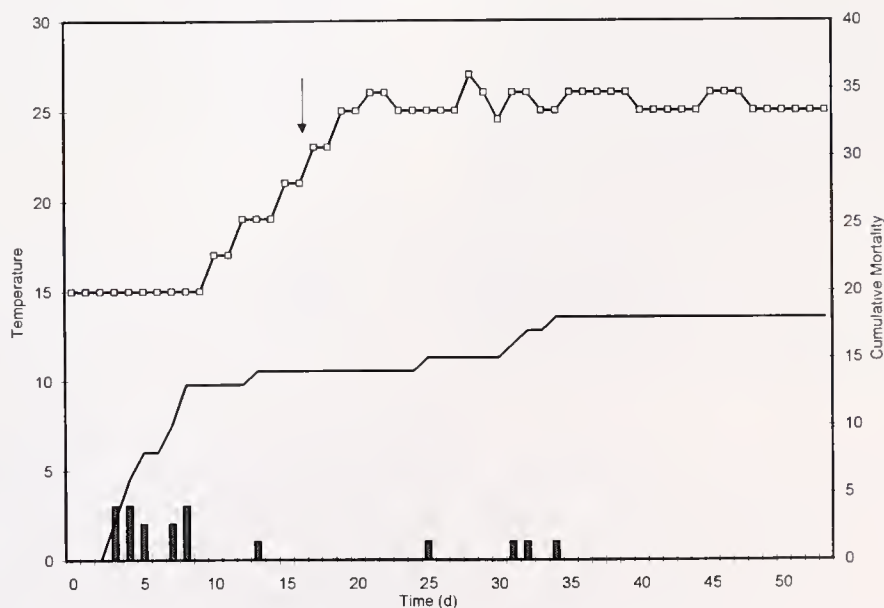


Figure 2. Water temperature (open squares) in recirculating system over the 8-wk experiment. Daily (shaded columns) and cumulative mortality (line) are indicated. The date (day 16) of highest ammonia level (4.20 mg/L) is indicated by an arrow.

shells were dried at 100°C for 48 h, dry weights were recorded, and the ratios were calculated.

Histologic slides were used for evaluation of digestive diverticula atrophy based on the methods of Winstead (1995). The area of the slide containing the digestive gland was divided into four quadrants, and five tubules were measured from each quadrant, for a total of 20 measurements from each oyster. Evaluations were done with 100× phase contrast microscopy using image analysis software (Optimas® 5.1a, Bioscan, Inc., Edmunds, WA). The total tubule area and the tubule lumen area were outlined by hand with a computer pointer (mouse), and the ratio of lumen area to total area was calculated and recorded in spreadsheet software (Microsoft Excel®, Microsoft Corp., Roselle, IL). A diverticula score was calculated for each oyster as the average of these 20 ratios, and a mean diverticula score was calculated for each sample.

Statistical Methods

Data were analyzed with the General Linear Models procedure (SAS Inc., Cary, NC). One-factor analysis of variance (ANOVA) was used to compare among mean values of weighted incidence of *P. marinus* infection, gametic stages, dry tissue-to-dry shell ratios, dry tissue-to-wet tissue ratios, and diverticula scores. A Duncan's Multiple Range Test was used to separate sample means. A significance level of $p < .05$ was used in all statistical analyses.

RESULTS

Water Quality

Water quality remained within the desired ranges throughout the study (Table 1). The system maintained temperatures within 1°C of the target temperatures (Fig. 2). However, transient increases in ammonia concentration were observed in weeks 2 and 3 as the temperature was increased (Fig. 2). The highest ammonia concentration was recorded on day 16 (4.2 mg/L). By day 21, concentrations had declined (0.2 mg/L) and remained negligible throughout the rest of the experiment.

Mortality and Disease

Mortality and *P. marinus* infection were low throughout the experiment. Of the 300 oysters stocked in the system, 13 deaths occurred during the acclimation period (week 1), followed by 5 deaths over the next 7 wks (Fig. 2). The weighted incidence of *P. marinus* infection did not exceed 1.00 (lowest level of infection) during the study. However, significant differences in weighted incidence of infection were noted among samples ($p < .017$) (Fig. 3). The highest observed weighted incidence of infection (0.70) was found in the week 7 field sample, followed by the week 7 laboratory sample (0.65). The lowest observed level of infection (0.10) was from the week 1 sample. During the study, 12 of the 18 oysters that died were examined for *P. marinus* infection. Of these, 2 were found to be infected, and each had an infection level of 1 (1 to 10 hyphospores per sample) (Table 3).

Gonad Development

Gametogenesis proceeded in the oysters during the experiment. At week 1, all of the gamete scores in the oysters sampled (29 of 29) were Stage 2 or lower (immature or developing). By week 8, 73% (22 of 30) of oysters sampled in the laboratory were at Stages 4 or 5 (mature) (Table 4). However, 93% (28 of 30) of field oysters sampled in week 8 were at Stages 4 or 5. The mean gametic stage increased through time, and significant differences were found ($p < .0001$) (Fig. 3). For all samples, the lowest mean value

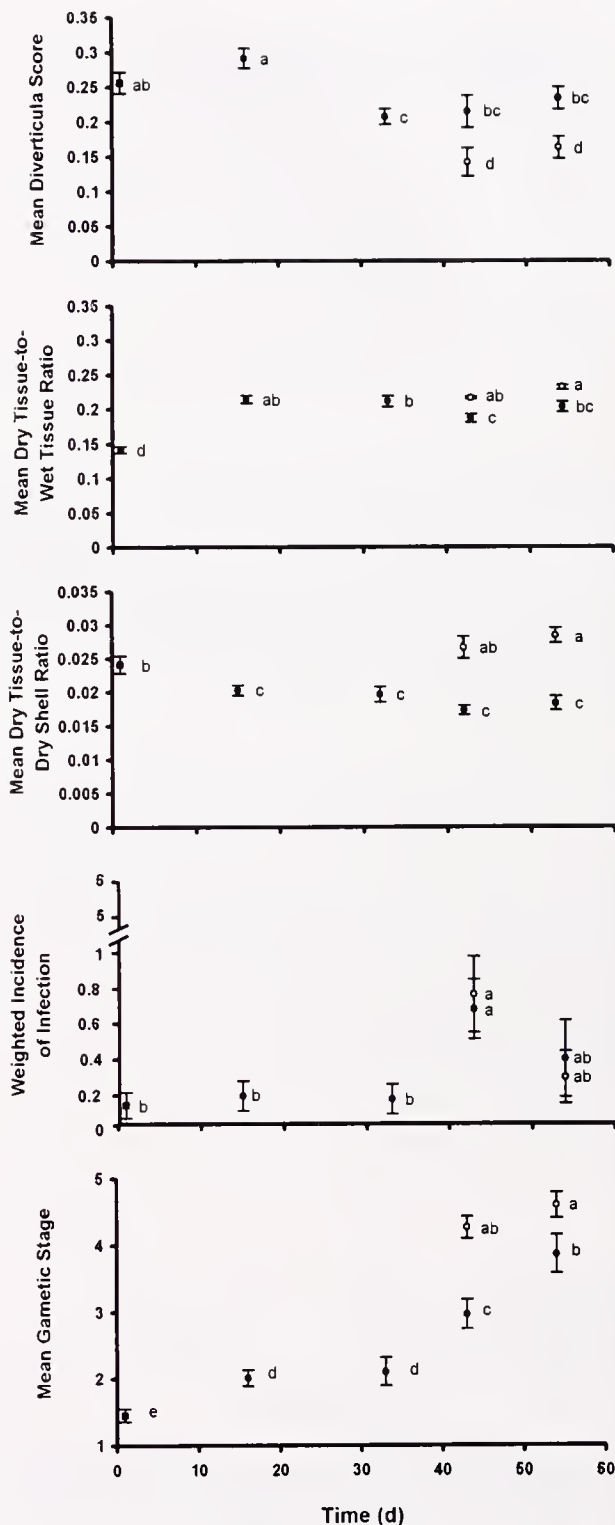


Figure 3. Weighted incidence of *Perkinsus marinus* infection, mean gametic stages, mean ratios of dry tissue weight-to-dry shell weight, mean ratios of dry tissue weight-to-wet tissue weight, and mean diverticula scores, over the 8-wk experiment. Initial samples are indicated with a square (these oysters were separated into laboratory and field samples). Laboratory samples are indicated with a closed circle. Field controls are indicated with an open circle. Bars indicate ± 1 SE. Within each panel, points sharing letters were not significantly different ($p > .05$).

TABLE 3.
Perkinsus marinus infection in *Crassostrea virginica* samples during an 8-wk study^a

Week	Source ^b	n	Individual Level of Infection							Prevalence	Intensity	Mean
			0	1	2	3	4	5	6			
1	F	20	18	2	0	0	0	0	0	10%	1.0	0.10
3	L	20	17	3	0	0	0	0	0	15%	1.0	0.15
5	L	20	18	1	1	0	0	0	0	10%	1.5	0.15
7	L	20	10	7	3	0	0	0	0	50%	1.3	0.65
7	F	20	11	5	3	1	0	0	0	45%	1.6	0.70
8	L	20	16	3	0	0	1	0	0	20%	1.8	0.35
8	F	20	17	1	2	0	0	0	0	15%	1.7	0.25
Mortalities	L	12	10	2	0	0	0	0	0	17%	1.0	0.167

^a Included is individual level of infection, prevalence (% infected oysters), infection intensity (mean level of infection among infected oysters), and weighted incidence of infection (mean level of infection for the entire sample) for each sample. At the foot of the table, all mortalities tested during the study are combined in one sample.

^b F, field sample; L, laboratory sample.

(1.4) was observed in the initial sampling (week 1), and the highest mean value (4.6) in the field sample at week 8. In the laboratory, a high value of 3.9 was observed in week 8. Gametic stage was significantly higher in each of the field samples than in the corresponding laboratory samples (Fig. 3).

Physiological Condition

Mean dry tissue-to-dry shell scores were significantly different among samples ($p < .0001$), with field samples higher than laboratory samples, and laboratory samples decreased after the first week (Fig. 3). The highest ratio (0.0284) was from the week 8 field sample. The week 1 initial ratio was 0.0241. Laboratory values after week 1 were not significantly different (Table 4).

Mean dry tissue-to-wet tissue ratios were significantly different among samples ($p < .0001$) (Fig. 3). The field samples from weeks 7 (0.216) and 8 (0.231) were highest. The laboratory values from weeks 7 and 8 were significantly lower than the corresponding field values (Fig. 3). The lowest laboratory value (0.141) was from the initial sampling at week 1.

The mean diverticula scores from each sample were significantly different ($p < .0001$). Scores of the field samples on weeks 7 and 8 were significantly lower than any score obtained for laboratory samples (Fig. 3). The lowest observed score (0.142) was from the week 7 field sample, and the highest (0.292) from the week 3 laboratory sample.

DISCUSSION

The goal of this project was to develop techniques for holding and conditioning of *C. virginica* in a recirculating system. To achieve this, water quality, levels of disease and mortality, gametogenesis, and physiologic condition were monitored over an 8-wk period. Although oysters have been held for extended periods in recirculating systems (Epifanio and Mootz 1976, Macmillan et al. 1994), to our knowledge, there are no reports of manipulation of gonadal maturation in such systems.

Water quality remained within desired ranges throughout the study. The required control of temperature for broodstock conditioning was achieved. However, ammonia concentrations approached stressful levels during weeks 2 and 3, as the temperature was increased to 25°C. It is likely that bacterial recolonization of the biologic filter was unable to keep pace with increased metabolic activity of the oysters, allowing nitrogenous wastes to accumulate. Five days after peaking on day 16, ammonia levels decreased to almost undetectable levels. With a higher stocking density (greater than 8.3 oysters/L), ammonia concentration could have reached a stressful level.

Although infections by *P. marinus* can cause extensive mortalities in *C. virginica*, and can reduce reproduction and physiologic condition (Hoffman et al. 1995, Kennedy et al. 1995), levels of this parasite were low throughout the experiment. The occur-

TABLE 4.
Gonadal development of *Crassostrea virginica* during the 8-wk study. Included is sample source and sample size, sex, number of individuals at each stage of development (1–6), and mean gametic stage for the entire sample

Week	Source ^a	n	Sex ^b				Gametic Stage						Mean
			M	F	H	U	1	2	3	4	5	6	
1	F	29	2	11	0	16	16	13	0	0	0	0	1.45
3	L	30	3	22	0	5	5	21	3	1	0	0	2.00
5	L	29	1	17	1	11	11	11	3	4	1	0	2.10
7	L	30	2	22	0	5	5	2	6	12	1	0	2.97
7	F	30	7	22	0	1	1	2	0	15	13	0	4.47
8	L	30	3	21	0	6	6	0	1	9	13	1	3.87
8	F	30	9	18	1	2	2	0	0	4	24	0	4.60

^a F, field sample; L, laboratory sample.

^b M, male; F, female; H, hermaphrodite; U, unidentifiable.

rence of infected individuals in Gulf Coast populations of *C. virginica* can be 100% (Craig et al. 1989). However, the initial infection incidence of *P. marinus* in the oysters in this experiment was 10% (Table 3). Although the technique used here to diagnose infection was less sensitive than other techniques (e.g., full body burden), especially with light levels of infection, high levels of infection would be obvious (Bushek et al. 1994). The mean level of infection for all samples remained less than 1.0 (lowest level of infection) for the entire 8 wks of the study. No mortalities during the experiment appeared to be due to *P. marinus* infection. Most mortalities (13) occurred during week 1 before the temperature was raised, and were presumably due to stress from harvesting, storage, transport, and stocking. The low level of mortalities (18 of 300 oysters stocked) and *P. marinus* infection suggest that other disease problems and severe stress due to poor nutrition or water quality were not present.

In healthy eastern oysters, gametogenesis would be expected to proceed at a water temperature of 25°C, and during the study, the majority of the oysters in the system reached maturity within 8 wks. Because gametogenesis in oysters in coastal waters begins during the time covered in this study (Shumway 1996), development was expected in the field controls. However, at the end of the study, oysters from the field control had a higher mean level of development. Nutrition is important in broodstock conditioning (Robinson 1992, Munanaka and Lannan 1984), and it is probable that oysters in natural waters obtained superior nutrition. Moreover, because artificial salt water was not replaced in the laboratory system, the oysters in the natural environment may have benefited from nutrients lacking or depleted in the laboratory. It should be noted that a mixture of algal species fed to oysters enhanced long-term growth in a recirculating system (Epifanio and Mootz 1976), whereas the oysters in our study were fed only a single algal species (resuspended from paste) at any time.

Factors causing oysters to utilize energy reserves, such as starvation, disease, spawning, or elevated temperatures, can cause a decrease in tissue weights and lower dry tissue-to-dry shell ratios (Mann 1978, Gabbot and Walker 1971). In this study, this condition index in the laboratory was reduced after week 1. The oysters came into the laboratory in good condition, remained in good condition in the field, but declined in the laboratory. Several explanations are possible. Spawning (Lucas and Beninger 1985) and *P. marinus* infection (Paynter and Bureson 1991) can cause decreases in dry tissue-to-dry shell ratios. Because little (if any) spawning occurred in the laboratory and field populations, and *P. marinus* infection was low in each, the likely causes for the poorer condition in the laboratory were temperature and nutrition. After week 2, oysters in the laboratory were exposed to an average temperature of ~25°C. A temperature-related increase in metabolic demand coupled with potentially poorer nutrition in the laboratory could have reduced condition. Previous studies with *Ostrea edulis* maintained for 4 wks in flow-through systems showed decreased condition compared with field oysters, attributable to higher temperature and less available food (Gabbot and Walker 1971).

Differences were observed when the ratio of dry tissue-to-wet

tissue was considered as an indicator of physiologic condition. A high proportion of water in tissues (and the associated low ratio of dry tissue:wet tissue) indicates a state of depleted energy reserves, possibly from starvation, disease, or winter conditions (Lucas and Beninger 1985). The oysters apparently suffered stress before stocking, resulting in a decreased ratio in the initial sample (week 1). Oysters recovered in the system and ratios increased in the following wks (although ratios decreased slightly in weeks 7 and 8, they remained significantly higher than in the week 1 sample). The ratios from the field samples of weeks 7 and 8 were higher than in the laboratory samples from those wks. A decrease in this ratio has been reported for *C. virginica* in a food-limited environment (Rheault and Rice 1996), and nutritional problems in the laboratory could have caused the differences observed between the field and laboratory samples. The decrease in weeks 7 and 8 in the laboratory was associated with a shift in the species of algae being fed to the oysters. For the last 2 wks of the study, *Chaetoceros calcitrans* replaced *Isochrysis galbana* as the food source, possibly causing the observed decreases.

The final measurement of physiologic condition was based on morphology of digestive diverticula. This technique has been correlated with starvation or salinity stress, and provides a general indicator of stress in oysters (Winstead 1995). The tubule ratios from weeks 1 and 3 were high as the oysters became acclimated to the increased temperature and artificial environment. Scores then became significantly lower, indicating reduced stress on the oysters in the laboratory, and possibly acclimation to the laboratory environment. However, scores obtained from the field samples were significantly lower than the laboratory samples in weeks 7 and 8. These scores agreed with the other measures of condition and suggested that the oysters in the laboratory were more stressed than those in the field. Again, a probable explanation is that field oysters received a superior diet.

In this study, gamete development was obvious in oysters held in the recirculating system with temperature manipulation. Water quality was maintained despite temperature changes, and mortality and disease were not problems. Oysters from field controls achieved a higher level of gametic development in 8 wks, and had a better physiologic condition, perhaps due to a more complete diet. This indicates a need for nutrition improvement, but demonstrates that broodstock conditioning of *C. virginica* is possible in a closed, recirculating system.

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GENETIC CHARACTERIZATION OF WILD AND SUSPENSION-CULTURED BLUE MUSSELS (*MYTILUS EDULIS* LINNEAUS, 1758) IN THE MAGDALEN ISLANDS (SOUTHERN GULF OF ST. LAWRENCE, CANADA)

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ABSTRACT The genetic characteristics of wild and suspension-cultured blue mussels from three lagoons of the Magdalen Islands were determined from the study of seven enzyme loci. Only 3.5% of mussels scored at the *MPI** locus were identified as *Mytilus trossulus*. For *M. edulis*, no major differences could be detected in allelic frequencies of wild mussels within and among the lagoons, indicating that gene flow around the Magdalen Islands is high. High levels of gene flow are also inferred from the high F_{st} and N_{em} values. Temporal stability is suggested by homogeneity in allelic frequencies of mussels from different size classes (assumed to represent different cohorts) sampled within each lagoon. An important heterozygote deficiency was observed in wild mussels from the House Harbour and the Great Entry lagoons where the mean H_o was 0.34 and 0.32, respectively. In contrast, there were no significant heterozygote deficiencies or excesses at most loci in wild mussels from Amherst Basin whose $H_o = 0.50$. The degree of heterozygosity was significantly higher in the wild mussels from Amherst Basin than in those from the other two lagoons. Significant decreases in the degree of heterozygosity were observed in suspension-cultured mussels 1 and 2 y after their transfer as spat from Amherst Basin to grow-out sites in the Great Entry and the House Harbour lagoons. One possible explanation for the observed decreases in the degree of heterozygosity is that a substantial number of heterozygotes collected in Amherst Basin may be lost through fall-off during suspension-culture activities, as suggested by the lower mean heterozygosities of sleeved mussels at both grow-out sites (mean $H_o = 0.35$ – 0.38).

KEY WORDS: *Mytilus edulis*, *Mytilus trossulus*, allozymes, allelic frequencies, heterozygosity, gene flow

INTRODUCTION

The Gulf of St. Lawrence is a well-known area where the distribution of *Mytilus edulis* and *M. trossulus* overlaps and hybridization occurs (Gosling 1992b). Although *M. trossulus* has been reported in numerous locations along the Gulf of St. Lawrence and the Atlantic coasts of Nova Scotia and Newfoundland (Varvio et al. 1988, McDonald et al. 1991, Bates and Innes 1995, Mallet and Carver 1995), the information regarding its presence in the Magdalen Islands which are located in the Gulf is scarce (Tremblay et al. 1998c). The composition of mussel stocks from the lagoons of the Magdalen Islands may differ relatively to these species. As *M. trossulus* seems to be less productive than *M. edulis* for mussel culture (Freeman et al. 1992, Mallet and Carver

1995) and blue mussels are cultivated in the Magdalen Islands, we needed to improve our understanding of these species' distribution in the area.

A previous study using reciprocal transfers suggested the existence of genetic differentiation among populations of blue mussels in the Magdalen Islands (Myrand and Gaudreault 1995). This study showed that with one exception, populations collected from closely neighboring areas (~65 km between the most distant populations) were all susceptible to high summer mortalities. The most resistant stock came from Amherst Basin and experienced an overall mortality rate of 18% between June 1990 and November 1991 at all sites. In contrast, mussels from Great Entry and House Harbour lagoons showed overall mortality rates of 89% and 78%, respectively, over the same period. As these mussels were kept under the same environmental conditions, the observed differential mortality was attributed to genetic rather than to environmental factors. The results from several studies in other regions support such conclusion (Dickie et al. 1984, Mallet et al. 1987, Mallet and

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Carver 1989, Mallet et al. 1990, Sephton 1991, Fuentes et al. 1992).

It was proposed that variations in resistance to summer mortality among populations from the Magdalen Islands are related to differential metabolic responses resulting from differences in heterozygosities (Tremblay et al. 1998 a,b,c). The genotypic frequencies of suspension-cultured mussels from the resistant stock of Amherst Basin were generally in agreement with Hardy-Weinberg expectations whereas a significant heterozygote deficiency was observed for those from the susceptible stock of the Great Entry lagoon (Tremblay et al. 1998c). Tremblay et al. (1998c) also found a negative correlation between multiple-locus heterozygosity and maintenance requirements for individuals from these two stocks. They proposed that the higher degree of heterozygosity of the Amherst Basin mussels confers an energetic advantage to this stock. Further, Tremblay et al. (1998 a,c) observed that mussels from the susceptible stock had higher maintenance requirements than those from the resistant stock during periods of negative

energetic balance. The susceptible stock from the Great Entry lagoon also had a higher thermal sensitivity, a negative scope for growth, an increased reliance on protein catabolism, and an increased period of lysosomal activity during the first half of August (Tremblay et al. 1998a,b,c). These results could be explained by the higher energetic needs of the Great Entry stock and are in agreement with previous studies on mollusks indicating that more heterozygous individuals have a higher protein synthesis efficiency and a higher scope for growth than more homozygous individuals (see Hawkins 1991). Although, the universality of the heterozygosity-fitness relationship has been questioned (Houle 1989, Jorgensen 1992, Britten 1996), it has been suggested by many authors that a higher heterozygosity may translate into faster growth rates and longer survival, particularly in stressful conditions (Hawkins et al. 1986, Zouros and Foltz 1987, Hawkins et al. 1989, Volckaert and Zouros 1989, Beaumont 1991, Koehn 1991, Gosling 1992b, Mitton 1993, Bayne and Hawkins 1997). Despite the marked differences in heterozygosity observed between sus-

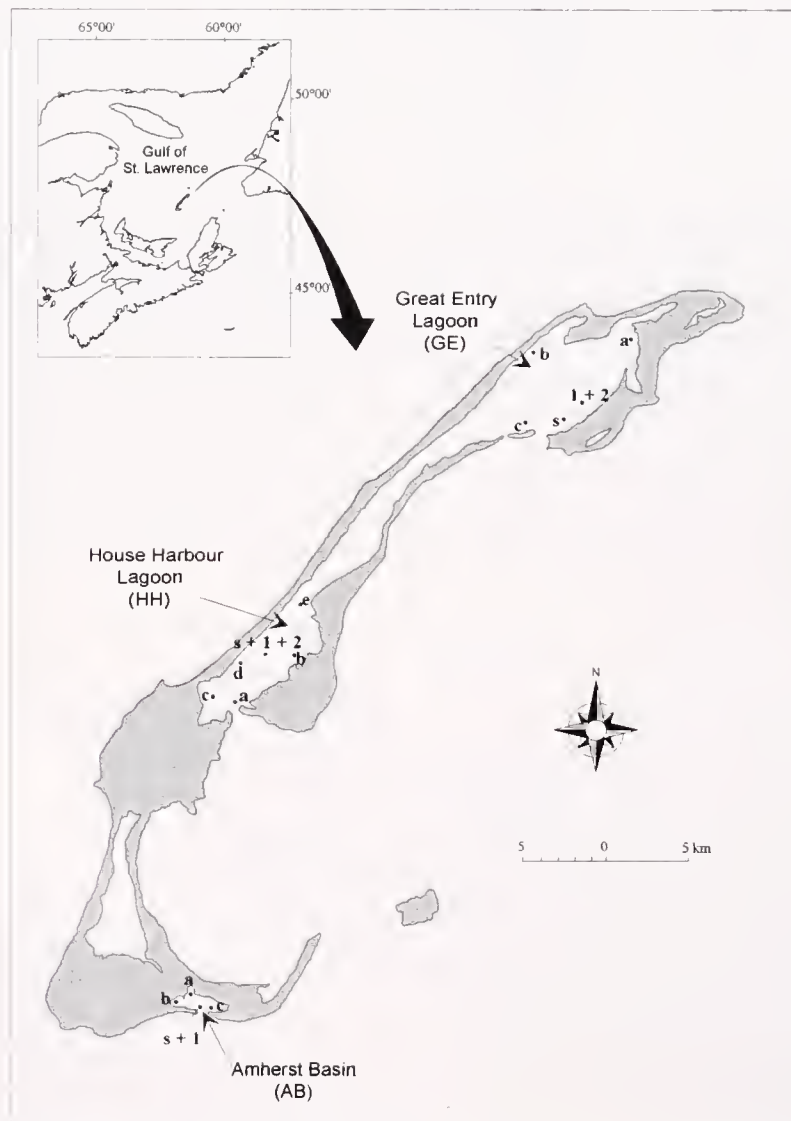


Figure 1. Map of the Magdalen Islands showing the sampling locations. Letters "a" to "e" refer to mussel beds in each lagoon. Letter "s" corresponds to the spat sampling locations, number "1" and "2" refer to mussels in suspension-culture for 1 and 2 y, respectively. Number "1" in Amherst Basin indicates the location of the mussels left undisturbed on collectors for 1 whole year.

pension-cultured mussels from two of the local stocks, Great Entry lagoon and Amherst Basin, (Tremblay et al. 1998c), the wild mussel populations of the Magdalen Islands have not been characterized genetically yet.

In this study, the genetic variation of wild mussels (including spat) from Amherst Basin, the Great Entry lagoon, and the House Harbour lagoon is described. Genetic variability is also measured on mussels from Amherst Basin which were sleeved and transferred as spat to growout sites in the Great Entry and the House Harbour lagoons for periods of 1 and 2 y. The specific objectives of this study were to (1) examine whether local stocks are formed with varying mixtures of *M. edulis* and *M. trossulus*, (2) test whether local wild stocks are genetically differentiated despite their proximity, and (3) compare the genetic variability of wild and suspension-cultured mussels. This study will provide a genetic basis to interpret past and future studies on blue mussels in the Magdalen Islands.

MATERIALS AND METHODS

Sampling

All mussels were sampled in October 1995 except when stated otherwise. Mussels were collected from all known wild beds: five beds in the House Harbor lagoon (HH-a to e), three in the Great Entry lagoon (GE-a to c), and three in Amherst Basin (AB-a to c) (Fig. 1). These samples consisted entirely of subtidal individuals since no intertidal populations are present in the Magdalen Islands.

Taken together, the samples from mussel beds in each lagoon likely included several cohorts because of the large size range observed (Table 1). Spat (3 to 4-mo-old) were collected in each lagoon on artificial collectors immersed in June 1995 and harvested in October 1995. In addition, we also sampled young mussels left undisturbed on artificial collectors in Amherst Basin during 1 y. Finally, we sampled suspension-cultured mussels originating from Amherst Basin which were transferred as spat to growout sites in the House Harbour and the Great Entry lagoons in late fall. These suspension-cultured mussels were provided by the local mussel growers and sampled 1 and 2 y after their transfer to the growout sites. The samples of suspension-cultured mussels in the Great Entry lagoon were obtained in July 1996 instead of October 1995, as were all the other samples. Therefore, the 1- and 2-y-old suspension-cultured mussels sampled in the House Harbour lagoon were transferred in fall 1993 and 1994, respectively; those sampled in the Great Entry lagoon were transferred in fall 1994 and 1995.

The samples collected from the mussel beds within each lagoon could have been pooled to get a larger sample size. Indeed, it can be assumed that the small size of the lagoons, especially Amherst Basin (~3 km²), and the prevailing water circulation patterns (Koutitonsky and Booth 1996) would favor panmixis within each lagoon. However, all samples were analyzed individually to detect any possible genetic heterogeneity caused by spatial microstructure or by temporal Wahlund effect. Approximately 90 individuals were used to characterize the wild mussels in each lagoon whereas

TABLE 1.

Sampled groups, sample size, shell length (mean \pm SD) and the relative frequency of *Mytilus edulis*, *M. trossulus* and their hybrids.

Location	Sample Size	Shell Length (cm)	<i>M. trossulus</i> (%)	<i>M. edulis</i> (%)	Hybrids (%)
Amherst Basin (AB):					
Mussel bed (AB-a)	33	38.9 \pm 3.5 ^c	2.8	97.2	0
Mussel bed (AB-b)	39	62.0 \pm 7.6 ^a	4.9	95.1	0
Mussel bed (AB-c)	23	52.0 \pm 4.1 ^b	3.7	96.3	0
Spat (AB-s)	58	36.1 \pm 4.2	1.5	97	1.5
One-year-old mussels left on collectors (AB-1)	47	57.5 \pm 2.6	3.9	96.1	0
House Harbour lagoon (HH):					
Mussel bed (HH-a)	11	54.1 \pm 4.7 ^a	7.6	92.4	0
Mussel bed (HH-b)	16	44.9 \pm 3.8 ^b	5.3	94.7	0
Mussel bed (HH-c)	14	37.8 \pm 3.2 ^c	5.9	94.1	0
Mussel bed (HH-d)	22	45.4 \pm 1.9 ^b	0	100	0
Mussel bed (HH-e)	25	39.0 \pm 2.7 ^c	0	100	0
Spat (HH-s)	46	31.5 \pm 3.0	2	96	2
Mussels in suspension-culture for 1 y (ABHH-1)	46	54.0 \pm 2.5	2	98	0
Mussels in suspension-culture for 2 y (ABHH-2)	46	54.8 \pm 4.0	2	98	0
Great Entry lagoon (GE):					
Mussel bed (GE-a)	30	50.7 \pm 5.0 ^b	5.8	94.2	0
Mussel bed (GE-b)	29	45.3 \pm 5.7 ^c	3.3	96.7	0
Mussel bed (GE-c)	28	64.2 \pm 5.9 ^a	3.3	96.7	0
Spat (GE-s)	47	28.6 \pm 1.3	0	100	0
Mussels in suspension-culture for 1 y (ABGE-1)	42	45.8 \pm 3.9	8.3	91.7	0
Mussels in suspension-culture for 2 y (ABGE-2)	47	58.6 \pm 6.1	8.2	89.8	2

Location abbreviations correspond to those used in Figure 1. Sample sizes correspond to the number of *M. edulis* individuals for which data were collected on all seven enzyme loci and shell length was measured. These data were used in the determination of the degree of heterozygosity. These figures represent the smallest possible sample size as larger numbers may have been analyzed at specific loci. Shell length of *M. edulis* mussels from wild beds was compared by ANOVAs in each lagoon. Values from a given lagoon followed by different letters (a,b,c) are significantly different (Tukey test $p < .05$).

TABLE 2.

Allelic frequencies, observed (H_o) and expected (H_e) heterozygosities at 7 polymorphic loci for the sampling sites in Amherst Basin (AB), the Great Entry lagoon (GE), and the House Harbour (HH) lagoon.

Locus/ Allele	Amherst Basin				Great Entry Lagoon				House Harbour Lagoon					
	AB-a	AB-b	AB-c	AB-s	GE-a	GE-b	GE-c	GE-s	HH-a	HH-b	HH-c	HH-d	HH-e	HH-s
<i>EST-1*</i>														
A	0.27	0.28	0.17	0.26	0.23	0.40	0.20	0.25	0.32	0.29	0.19	0.33	0.38	0.26
B	0.30	0.28	0.21	0.32	0.22	0.07	0.32	0.18	0.27	0.12	0.19	0.15	0.16	0.22
C	0.39	0.41	0.50	0.36	0.53	0.48	0.45	0.53	0.41	0.59	0.50	0.43	0.44	0.47
D	0.03	0.03	0.12	0.06	0.02	0.05	0.04	0.04	0.00	0.00	0.12	0.09	0.02	0.05
H_o	0.70	0.59	0.59	0.60	0.38	0.41	0.29	0.53	0.09	0.35	0.37	0.43	0.36	0.27
H_e	0.69	0.68	0.68	0.70	0.62	0.61	0.67	0.63	0.69	0.57	0.66	0.69	0.65	0.67
D	0.01	-0.13	-0.14	-0.14	-0.40*	-0.32	-0.57**	-0.15	-0.87**	-0.38*	-0.45*	-0.37	-0.45**	-0.60**
p	0.47	0.71	0.91	0.10	0.03	0.08	0.001	0.92	0.004	0.02	0.011	0.13	0.008	<0.001
n	33	39	17	58	32	29	28	47	11	17	16	23	25	48
<i>EST-2*</i>														
A	0.18	0.28	0.23	0.33	0.25	0.22	0.23	0.26	0.36	0.35	0.25	0.18	0.32	0.30
B	0.55	0.41	0.57	0.44	0.42	0.62	0.47	0.51	0.51	0.47	0.53	0.61	0.58	0.48
C	0.27	0.31	0.20	0.23	0.33	0.15	0.30	0.23	0.13	0.18	0.22	0.21	0.10	0.22
H_o	0.52	0.67	0.40	0.57	0.34	0.21	0.21	0.50	0.36	0.12	0.31	0.23	0.44	0.35
H_e	0.60	0.66	0.59	0.65	0.66	0.55	0.65	0.63	0.63	0.64	0.61	0.56	0.56	0.63
D	-0.15	0.01	-0.33	-0.13	-0.48**	-0.62**	-0.67**	-0.20	-0.42**	-0.82**	-0.50**	-0.59*	-0.22	-0.43**
p	0.83	0.71	0.27	0.12	<0.001	0.002	0.002	0.35	0.004	0.001	0.009	0.011	0.16	0.005
n	33	39	30	58	32	29	28	48	11	17	16	22	25	52
<i>GPI*</i>														
A	0.19	0.18	0.12	0.13	0.14	0.05	0.04	0.14	0.08	0.18	0.06	0.04	0.10	0.12
B	0.03	0.01	0.04	0.00	0.02	0.02	0.04	0.00	0.00	0.03	0.00	0.00	0.04	0.00
C	0.17	0.23	0.19	0.18	0.18	0.17	0.27	0.25	0.04	0.18	0.22	0.22	0.18	0.18
D	0.43	0.32	0.35	0.40	0.41	0.48	0.57	0.40	0.58	0.47	0.47	0.52	0.46	0.42
E	0.04	0.02	0.04	0.03	0.00	0.07	0.02	0.00	0.00	0.06	0.00	0.02	0.00	0.01
F	0.14	0.23	0.27	0.26	0.27	0.21	0.07	0.21	0.29	0.09	0.25	0.20	0.22	0.27
H_o	0.69	0.49	0.58	0.71	0.56	0.41	0.25	0.32	0.33	0.53	0.51	0.56	0.56	0.48
H_e	0.74	0.76	0.77	0.73	0.71	0.69	0.60	0.72	0.59	0.72	0.67	0.64	0.71	0.71
D	-0.07	-0.36**	-0.25**	-0.03	-0.23*	-0.41**	-0.59**	-0.56**	-0.43*	-0.27	-0.27	-0.14	-0.21	-0.46**
p	0.63	0.004	0.009	0.50	0.04	0.001	<0.001	<0.001	0.015	0.18	0.11	0.12	0.052	0.004
n	35	41	26	61	32	29	28	50	12	17	16	23	25	48
<i>LAP*</i>														
A	0.43	0.44	0.39	0.40	0.42	0.55	0.45	0.44	0.46	0.56	0.44	0.33	0.48	0.48
B	0.18	0.23	0.28	0.25	0.22	0.26	0.14	0.22	0.25	0.06	0.16	0.35	0.24	0.19
C	0.32	0.33	0.33	0.33	0.36	0.19	0.36	0.34	0.29	0.38	0.40	0.32	0.28	0.33
D	0.07	0.00	0.00	0.02	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H_o	0.56	0.56	0.65	0.62	0.41	0.31	0.43	0.28	0.42	0.29	0.38	0.39	0.24	0.27
H_e	0.69	0.65	0.67	0.67	0.65	0.60	0.66	0.65	0.67	0.55	0.62	0.67	0.63	0.63
D	-0.19	-0.14	-0.03	-0.08	-0.38	-0.48*	-0.36**	-0.57**	-0.38	-0.47*	-0.41	-0.43*	-0.63**	-0.57**
p	0.17	0.85	0.22	0.37	0.30	0.013	0.001	0.002	0.48	0.033	0.28	0.031	0.007	<0.001
n	34	39	23	65	32	29	28	50	12	17	16	23	25	48
<i>MPI*</i>														
A	0.15	0.26	0.31	0.22	0.06	0.14	0.21	0.24	0.25	0.32	0.12	0.11	0.10	0.25
B	0.72	0.70	0.67	0.76	0.86	0.76	0.75	0.74	0.71	0.68	0.66	0.87	0.88	0.73
C	0.13	0.04	0.02	0.02	0.08	0.10	0.04	0.02	0.04	0.00	0.22	0.02	0.02	0.02
H_o	0.44	0.44	0.46	0.40	0.28	0.28	0.36	0.28	0.42	0.41	0.31	0.26	0.24	0.46
H_e	0.45	0.45	0.58	0.37	0.25	0.40	0.40	0.40	0.45	0.45	0.51	0.23	0.22	0.41
D	-0.01	-0.01	-0.25	0.06	0.11	-0.31	-0.10	-0.30*	-0.08	-0.09	-0.41	0.10	0.09	0.12
p	0.64	0.83	0.14	0.74	0.39	0.15	0.72	0.044	0.80	0.71	0.17	0.52	0.54	0.30
n	34	39	26	63	32	29	28	50	12	17	16	23	25	48
<i>ODH*</i>														
A	0.00	0.08	0.06	0.08	0.09	0.07	0.02	0.02	0.04	0.06	0.00	0.02	0.04	0.03
B	0.96	0.84	0.75	0.79	0.80	0.76	0.89	0.90	0.92	0.85	0.72	0.85	0.92	0.88
C	0.04	0.08	0.19	0.13	0.11	0.17	0.09	0.08	0.04	0.09	0.28	0.13	0.04	0.09
H_o	0.88	0.26	0.35	0.25	0.28	0.28	0.07	0.02	0.17	0.29	0.31	0.30	0.08	0.21
H_e	0.86	0.28	0.40	0.36	0.35	0.40	0.20	0.18	0.16	0.27	0.42	0.26	0.15	0.23
D	0.03	-0.07	-0.14	-0.30*	-0.19	-0.31	-0.64**	-0.35*	0.02	0.09	-0.25	0.13	-0.48**	-0.08
p	0.83	0.86	0.62	0.016	0.40	0.15	<0.001	0.012	0.83	0.53	0.29	0.43	0.008	0.68
n	34	39	26	63	32	29	28	50	12	17	16	23	25	48

continued on next page

TABLE 2.

continued

Locus/ Allele	Amherst Basin				Great Entry Lagoon				House Harbour Lagoon					
	AB-a	AB-b	AB-c	AB-s	GE-a	GE-b	GE-c	GE-s	HH-a	HH-b	HH-c	HH-d	HH-e	HH-s
PGM*														
A	0.10	0.11	0.14	0.08	0.07	0.07	0.05	0.05	0.04	0.13	0.006	0.06	0.06	0.06
B	0.79	0.73	0.72	0.68	0.78	0.72	0.70	0.73	0.67	0.62	0.74	0.70	0.70	0.79
C	0.11	0.16	0.14	0.22	0.15	0.21	0.21	0.20	0.21	0.25	0.20	0.24	0.24	0.15
D	0.00	0.00	0.00	0.02	0.00	0.00	0.04	0.02	0.08	0.00	0.00	0.00	0.00	0.00
Ho	0.43	0.47	0.36	0.37	0.27	0.28	0.29	0.24	0.33	0.31	0.30	0.32	0.33	0.33
He	0.37	0.44	0.45	0.48	0.36	0.44	0.47	0.43	0.52	0.55	0.42	0.45	0.45	0.35
D	0.17	0.08	-0.20	-0.24	-0.27	-0.37**	-0.40*	-0.44**	-0.36	-0.43*	-0.28**	-0.31	-0.30	-0.05
p	0.12	0.46	0.25	0.21	0.066	0.007	0.023	<0.001	0.30	0.045	0.005	0.08	0.07	0.99
n	35	40	25	60	30	29	28	50	12	16	23	25	25	48
All loci														
Ho	0.49	0.50	0.50	0.50	0.36	0.31	0.27	0.32	0.31	0.33	0.36	0.36	0.32	0.33
He	0.52	0.56	0.58	0.57	0.52	0.54	0.52	0.52	0.53	0.54	0.57	0.51	0.49	0.52

D = coefficient of heterozygote deficiency or excess. p = probability of a significant excess or deficiency in heterozygotes.

* $p < .05$, ** $p < .01$.

45–60 mussels were used to describe the genetic characteristics of the spat and of the 1- and 2-y-old suspension-cultured mussels. No attempt was made to target mussels of a specific shell length. After sampling, the mussels were frozen and kept at -80°C until electrophoretic analyses.

Gel Electrophoresis

Mussels were thawed and their shell length was measured to the nearest mm. A small piece of the digestive gland was homogenized in an approximately equal volume of homogenization buffer (0.2 M Tris-HCl, pH 8.0, with 30% sucrose, 1% polyvinylpyrrolidone, 0.1% Nicotinamide Adenine Dinucleotide (NAD), 5 mM dithiothreitol, and 1 mM 4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride (Pefabloc SC, Boehringer Mannheim). The solution was centrifuged at 15,000 g for 30 min at 4°C and the supernatant was applied to a horizontal cellulose acetate gel (Hebert and Beaton 1989). The studied polymorphic enzymes were glucose phosphate isomerase (GPI EC 5.3.1.9), mannosephosphate isomerase (MPI, EC 5.3.1.8), phosphoglucosmutase (PGM, EC 2.7.5.1), octopine dehydrogenase (ODH, EC 1.5.1.11), and leucine aminopeptidase (LAP, 3.4.11). Esterases (EST, EC 3.1.1.1) were scored after migration on vertical discontinuous polyacrylamide slab gels (Ornstein 1964). Enzymes were stained according to Harris and Hopkinson (1976) and Hebert and Beaton (1989). A "standard" was prepared by mixing homogenates of individuals of different genotypes. This "standard" of all possible alleles for a given locus was applied on each gel to ensure exact allele identification. The gene nomenclature for protein-coding loci follows the recommendation of Shaklee et al. (1990).

The variability at the *MPI** locus was used to discriminate *Mytilus trossulus* from *M. edulis* according to the method used by Mallet and Carver (1995) and McDonald et al. (1991).

Statistical Analyses

Statistical tests were performed with the version 6.11 of the SAS package (1982) except stated otherwise. Frequency distributions of *M. edulis* and *M. trossulus* were compared with Fisher's exact tests for contingency tables because of low expected frequencies (Cody and Smith 1991). Shell length of the wild mussels

in each lagoon was compared with ANOVAs followed by a *posteriori* Tukey tests.

Allelic frequencies, observed and expected heterozygosities under the assumption of Hardy-Weinberg equilibrium, heterozygote deficiency index (*D*), and Wright's (1978) fixation index (F_{ST}) were calculated for each locus using the BIOSYS-1 program of Swofford and Selander (1989). Comparisons of allelic frequencies for each locus within and between lagoons were carried out with χ^2 Monte-Carlo simulations (Roff and Bentzen 1989) of the REAP program (McElroy et al. 1991).

The observed heterozygosities were first compared with paired *t*-tests (Archie 1985) but the power of these tests was too weak to find any possible significant differences (type II error). The results of these *t*-tests are not presented. However, the degree of heterozygosity defined as the mean number of heterozygous loci per individual (Zouros and Foltz 1987) was compared among different groups of mussels with Wilcoxon tests because data did not follow normal distributions. Only mussels with a complete data set for all seven loci were used in these tests. Sequential Bonferroni tests were used to maintain the overall significance level at $\alpha = 0.05$ within multiple comparisons carried out with Monte Carlo simulations and Wilcoxon tests (Rice 1989).

Gene flow and the number of migrants between the three lagoons were estimated from Wright's (1978) fixation index according to the formula:

$$F_{ST} = 1 / (1 + 4 N_e m)$$

where *m* = migration rate and N_e = effective number of individuals.

Finally, the absolute differentiation between different groups of mussels was estimated using Nei's genetic distances.

RESULTS

Frequency Distribution of *M. trossulus* and *M. edulis*

The presence of *M. trossulus* in the different samples was always $<10\%$ (Table 1), and only 3.5% of the 709 individuals scored at the *MPI** locus were assigned to this species and 0.4% were

TABLE 3.

Allelic frequencies, observed (H_o) and expected (H_e) heterozygosities at 7 polymorphic loci for mussels left undisturbed on collectors in Amherst Basin during 1 yr (AB-1) and also mussels taken from Amherst Basin and transferred as spat for suspension-culture to growout sites in the Great Entry and the House Harbour lagoons for 1 (ABGE-1 and ABHH-1) and 2 (ABGE-2 and ABHH-2).

Locus/ Alleles	Amherst Basin		Great Entry Lagoon				House Harbour Lagoon			
	AB-1	AB-Wild	ABGE-1	ABGE-2	ABGE	GE-Wild	ABHH-1	ABHH-2	ABHH	HH-Wild
<i>EST-1*</i>										
A	0.36	0.26	0.31	0.11	0.20	0.26	0.26	0.27	0.27	0.30
B	0.23	0.29	0.26	0.38	0.33	0.20	0.28	0.16	0.22	0.18
C	0.36	0.40	0.38	0.41	0.40	0.50	0.42	0.55	0.49	0.47
D	0.05	0.05	0.05	0.10	0.08	0.04	0.04	0.02	0.03	0.05
H_o	0.64	0.662	0.33	0.38	0.36	0.42	0.51	0.48	0.50	0.31
H_e	0.69	0.69	0.70	0.67	0.69	0.64	0.68	0.60	0.65	0.64
D	-0.08	-0.10	-0.52**	-0.44**	-0.48**	-0.34**	-0.25	-0.21	-0.24	-0.51**
p	0.54	0.33	<0.001	0.006	<0.001	0.001	0.36	0.38	0.17	<0.001
n	47	147	42	50	92	136	49	48	97	140
<i>EST-2*</i>										
A	0.25	0.27	0.43	0.29	0.36	0.25	0.35	0.30	0.32	0.29
B	0.58	0.48	0.43	0.54	0.49	0.50	0.39	0.36	0.38	0.52
C	0.17	0.25	0.14	0.17	0.16	0.25	0.26	0.34	0.30	0.19
H_o	0.42	0.52	0.29	0.28	0.28	0.34	0.48	0.46	0.47	0.31
H_e	0.58	0.62	0.62	0.60	0.61	0.62	0.66	0.67	0.67	0.61
D	-0.28*	-0.14	-0.54**	-0.53**	-0.54**	-0.45**	-0.28**	-0.32*	-0.30**	-0.48**
p	0.02	0.08	<0.001	<0.001	<0.001	<0.001	0.002	0.04	<0.001	<0.001
n	48	160	49	50	99	137	46	46	92	143
<i>GPI*</i>										
A	0.14	0.15	0.09	0.08	0.09	0.10	0.12	0.07	0.10	0.10
B	0.02	0.02	0.03	0.04	0.04	0.01	0.01	0.00	0.01	0.01
C	0.14	0.19	0.18	0.15	0.17	0.22	0.23	0.18	0.20	0.18
D	0.38	0.38	0.33	0.45	0.39	0.45	0.40	0.47	0.43	0.47
E	0.03	0.03	0.05	0.08	0.07	0.04	0.08	0.04	0.06	0.01
F	0.29	0.23	0.31	0.19	0.25	0.17	0.16	0.24	0.20	0.23
H_o	0.61	0.66	0.58	0.51	0.55	0.42	0.33	0.35	0.34	0.50
H_e	0.74	0.77	0.76	0.73	0.75	0.71	0.75	0.69	0.72	0.69
D	-0.17	-0.16**	-0.23**	-0.30**	-0.27**	-0.40**	-0.57**	-0.50**	-0.53**	-0.27**
p	0.056	0.003	0.05	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
n	49	163	48	49	97	139	49	49	98	141
<i>LAP*</i>										
A	0.41	0.41	0.16	0.05	0.11	0.46	0.46	0.44	0.45	0.46
B	0.24	0.24	0.29	0.47	0.38	0.21	0.21	0.19	0.20	0.21
C	0.35	0.33	0.51	0.45	0.48	0.32	0.31	0.34	0.32	0.33
D	0.00	0.02	0.04	0.03	0.03	0.01	0.02	0.03	0.03	0.00
H_o	0.71	0.62	0.61	0.64	0.62	0.34	0.47	0.48	0.47	0.31
H_e	0.65	0.66	0.63	0.58	0.62	0.64	0.65	0.65	0.66	0.64
D	0.08	-0.11	-0.04	0.09	0.01	-0.47**	-0.28*	-0.27	-0.28	-0.51*
p	0.54	0.49	0.53	0.92	0.17	<0.001	0.029	0.58	0.058	<0.001
n	49	161	51	47	98	139	49	48	97	141
<i>MPI*</i>										
A	0.26	0.23	0.09	0.12	0.11	0.17	0.16	0.25	0.20	0.20
B	0.73	0.72	0.74	0.81	0.77	0.77	0.82	0.71	0.77	0.76
C	0.01	0.05	0.17	0.07	0.12	0.06	0.02	0.04	0.03	0.04
H_o	0.37	0.43	0.20	0.23	0.23	0.29	0.37	0.37	0.37	0.20
H_e	0.40	0.42	0.41	0.33	0.38	0.37	0.31	0.43	0.37	0.30
D	-0.08	0.06	-0.52**	-0.25*	-0.40	-0.20	0.19	-0.15	-0.02	-0.05
p	0.63	0.22	0.001	0.017	<0.001	0.055	0.13	0.44	0.86	0.95
n	49	162	45	44	89	139	49	49	98	141
<i>ODH*</i>										
A	0.04	0.06	0.01	0.03	0.02	0.05	0.03	0.02	0.03	0.03
B	0.86	0.83	0.93	0.88	0.90	0.84	0.84	0.88	0.86	0.86
C	0.10	0.11	0.06	0.09	0.08	0.11	0.13	0.10	0.12	0.11
H_o	0.29	0.23	0.14	0.24	0.19	0.18	0.24	0.21	0.22	0.22
H_e	0.26	0.29	0.13	0.22	0.18	0.27	0.28	0.22	0.25	0.25
D	0.12	-0.21*	0.06	-0.10	0.08	-0.34**	-0.14	-0.08	-0.11	-0.11
p	0.26	0.014	0.62	0.36	0.31	<0.001	0.42	0.66	0.38	0.33
n	49	162	49	50	99	139	49	49	98	141

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TABLE 3.
continued

Locus/ Alleles	Amherst Basin		Great Entry Lagoon				House Harbour Lagoon			
	AB-1	AB-Wild	ABGE-1	ABGE-2	ABGE	GE-Wild	ABHH-1	ABHH-2	ABHH	HH-Wild
<i>PGM*</i>										
A	0.09	0.10	0.07	0.05	0.06	0.06	0.05	0.07	0.06	0.07
B	0.76	0.72	0.70	0.78	0.74	0.73	0.78	0.72	0.75	0.72
C	0.15	0.17	0.21	0.17	0.19	0.19	0.17	0.19	0.18	0.20
D	0.00	0.01	0.02	0.00	0.01	0.02	0.00	0.02	0.01	0.01
<i>H_o</i>	0.39	0.41	0.32	0.25	0.29	0.26	0.25	0.31	0.28	0.32
<i>H_e</i>	0.40	0.44	0.47	0.37	0.42	0.42	0.36	0.45	0.40	0.43
D	-0.04	-0.08	-0.31*	-0.31**	-0.31**	-0.38**	-0.31**	-0.30*	-0.30**	-0.25**
p	0.90	0.78	0.015	0.001	<0.001	<0.001	0.001	0.018	<0.001	<0.001
n	49	160	50	47	97	137	48	48	96	149
All loci										
<i>H_o</i>	0.49	0.50	0.35	0.36	0.36	0.32	0.38	0.38	0.38	0.34
<i>H_e</i>	0.53	0.56	0.53	0.50	0.52	0.53	0.53	0.53	0.53	0.52

ABGE and ABHH = pooled samples of 1- and 2-yr-old suspension-cultured mussels at the grow out sites. Wild = mussel beds + spat in each lagoon. D = coefficient of heterozygote deficiency or excess. p = probability of a significant excess or deficiency in heterozygotes.

* p < .05, **p < .01.

hybrids. The respective proportion of *M. trossulus* and *M. edulis* among wild mussels (wild beds and spat) within each lagoon was similar (Amherst Basin: $X^2 = 1.03$, $DF = 3$, $p = .79$; Great Entry lagoon: $X^2 = 2.71$, $DF = 3$, $p = .44$; House Harbour lagoon: $X^2 = 3.76$, $DF = 5$, $p = .58$). Further, we found no difference ($X^2 = 0.02$, $DF = 2$, $p = 1.0$) among the three lagoons according to their respective abundance of both species. The mean proportion of *M. trossulus* was 3.2% in Amherst Basin, 3.8% in the House Harbour lagoon, and 3.1% in the Great Entry lagoon. All *M. trossulus* individuals and hybrids were discarded from further analyses.

Shell Length

The mean shell length was significantly different ($p < .0001$) and highly variable between mussel beds within each lagoon (Table 1). For example, the mean shell length of mussels from Amherst Basin ranged from 38.9 ± 3.5 mm (AB-a) to 62.0 ± 7.6 mm (AB-b). A *posteriori* Tukey tests showed that samples collected from the various beds in the Great Entry lagoon as well as

those from Amherst Basin were all significantly different ($p < .05$) from each other. In the House Harbour lagoon, there were no significant differences ($p > .05$) between the HH-b and the HH-d beds or between the HH-c and HH-e beds.

Allelic Frequencies

The standard data on allelic frequencies for all groups of mussels are detailed in Tables 2 and 3. Allelic frequencies of wild mussels (mussel beds and spat) were compared for each locus within each lagoon. Differences were only detected at the *MPI** locus in both Amherst Basin and the House Harbour lagoon and at the *GPI** locus in the Great Entry lagoon (Table 4). When wild mussels data (mussel beds and spat) were combined, no significant differences could be found among the three lagoons (Table 4).

The allelic frequencies in 1- and 2-y-old suspension-cultured mussels held in the House Harbour lagoon were similar for all loci whereas a significant difference was observed at the *EST-1** locus only for those held at the growout site in the Great Entry lagoon

TABLE 4.

Multiple heterogeneity X^2 tests calculated with Monte-Carlo simulations to compare the allelic frequencies of wild mussels (mussel beds and spat) at each locus within each lagoon and also among the three lagoons.

Loci	Within Amherst Basin (3 Mussel Beds + Spat)			Within Great Entry Lagoon (3 Mussel Beds + Spat)			Within House Harbour Lagoon (5 Mussel Beds + Spat)			Among the three lagoons (Mussel Beds + Spat pooled)		
	X^2	DF	p	X^2	DF	p	X^2	DF	p	X^2	DF	p
<i>Est-1*</i>	12.67	9	.198	16.64	9	.050	15.87	15	.427	13.89	6	.022
<i>Est-2*</i>	8.28	6	.245	7.27	6	.290	7.83	10	.648	5.52	4	.221
<i>GPI*</i>	12.78	15	.628	31.72	15	.002*	27.43	25	.317	12.93	10	.227
<i>LAP*</i>	11.35	9	.254	18.95	9	.028	12.96	10	.233	9.11	6	.164
<i>MPI*</i>	18.49	6	.004*	14.71	6	.016	39.17	10	<.001*	3.29	4	.528
<i>ODH*</i>	14.38	6	.019	10.50	6	.102	15.90	10	.098	2.59	4	.633
<i>PGM*</i>	8.29	9	.520	5.06	9	.832	28.61	15	.019	6.81	6	.326

Sequential Bonferroni tests were used to keep the overall significance threshold $\alpha = 0.05$ for each group of multiple comparisons (each column).

* Indicates a significant difference at an overall $\alpha = 0.05$.

(Table 5). Thus, data from 1- and 2-y-old suspension-cultured mussels were pooled for each growout site. Allelic frequencies of wild mussels from Amherst Basin were similar to those of their congeners transferred for suspension-culture to the House Harbour lagoon but were significantly different from those transferred to the Great Entry lagoon at the *LAP** and *MPI** loci (Table 5).

Conformance to the Hardy-Weinberg Equilibrium

The proportions of observed heterozygotes over all loci (*H_o*) in wild mussels (mussel beds and spat) from Amherst Basin were generally in good agreement with values expected (*H_e*) under the Hardy-Weinberg equilibrium at 5 out of the 7 loci (Table 3). In contrast, those from the House Harbour and the Great Entry lagoons showed marked deviations from the Hardy-Weinberg expectations at 5 and 6 loci, respectively. The *H_o* values in Amherst Basin samples were 0.49–0.50 whereas the *H_e* varied between 0.52 and 0.58 (Table 2). In contrast, the *H_o* values for the different groups of wild mussels from the Great Entry lagoon varied from 0.27 to 0.36 and were much lower than the *H_e* values (0.52–0.54). The same trend was observed in the House Harbour lagoon where *H_o* varied between 0.31 and 0.36 compared with *H_e* values of 0.49–0.57.

Degree of Heterozygosity

The degree of heterozygosity was compared for six groups of mussels: wild mussels from Amherst Basin (AB-Wild), the House Harbour lagoon (HH-Wild) and the Great Entry lagoon (GE-Wild), suspension-cultured mussels taken as spat from Amherst Basin and transferred to growout sites in House Harbour (ABHH) and Great Entry (ABGE) lagoons, and mussels from Amherst Basin left undisturbed on collectors for 1 y (AB-1). The frequency distributions of the degree of heterozygosity (number of heterozygous loci per individual) for these groups are shown in Figure 2. Wild mussels from Amherst Basin had a higher degree of heterozygosity than the wild mussels from the House Harbour lagoon ($Z = -6.35$, $p = .0001$) and the Great Entry lagoon ($Z = -7.40$, $p = .0001$). However, wild mussels from both the House Harbour and the Great Entry lagoons had similar values ($Z = -0.83$, $p = .41$). No difference was found between wild mussels from Amherst Basin and those left on collectors in this lagoon ($Z = 0.34$, $p = .74$) or between suspension-cultured mussels in both lagoons (ABGE vs ABHH: $Z = 1.28$, $p = .20$). However, the wild mussels from

Amherst Basin had a higher degree of heterozygosity than their congeners transferred for suspension-culture to either the House Harbour ($Z = -3.43$, $p = .0006$) and the Great Entry lagoons ($Z = -4.87$, $p = .0001$).

Gene Flow and Genetic Distances

Values of $N_e m$ calculated for each locus were high for wild mussels from the three lagoons (Table 6). If we assume that the differences between loci are not due to natural selection, an overall value of 83 is estimated from the *F_{st}* mean value over all loci. Such high values suggest that considerable gene flow is taking place between the lagoons of the Magdalen Islands.

The Nei's unbiased genetic distances among the six groups of pooled samples (c.f. section on Degree of Heterozygosity) were small and varied between 0.000 and 0.035 (Table 7).

DISCUSSION

Mussels from the wild beds of the three lagoons did not differ according to their relative composition of *M. trossulus* and *M. edulis*. The occurrence of *M. trossulus* is very low in the Magdalen Islands and only 3.5% of all sampled mussels were assigned to this species. The same relative abundance of *M. trossulus* among mussel beds suggests that its occurrence was maintained at a low level over the years as the large range of shell lengths indicates the probable presence of several cohorts. This hypothesis is also supported by Tremblay et al. (1998c) who found only 2.4% of *M. trossulus* in samples collected in 1992 and 1993. The high water temperature (>20°C) reached in summer (Myrand 1991, Myrand and Gaudreault 1995) may partly explain the low occurrence of *M. trossulus* since this species on the Pacific coast of North America shows some signs of stress at temperatures as low as 13°C (Hoffman and Somero 1996). Further, *M. trossulus* is known to be a more northerly species than *M. edulis* (Gosling 1992a). However, the factors influencing the distribution patterns of both species in eastern Canada are not well understood yet (Bates and Innes 1995, Mallet and Carver 1995). The low occurrence of *M. trossulus* in the Magdalen Islands is in agreement with values reported from the neighboring Prince Edward Island and Northumberland Strait area (Mallet and Carver 1995). As observed in other studies (Freeman et al. 1994, Bates and Innes 1995, Mallet and Carver 1995), we found only few hybrids. The differential susceptibilities of the Magdalen Islands stocks to summer mortality (Myrand and Gaud-

TABLE 5.

Multiple heterogeneity χ^2 tests calculated with Monte-Carlo simulations to compare the allelic frequencies of suspension-cultured mussels transferred to growout sites for 1 and 2 y and wild mussels from Amherst Basin (AB-Wild) to those coming from this lagoon and transferred as spat to growout sites in the Great Entry (ABGE) and the House Harbour (ABHH) lagoons.

Loci	ABHH-1 vs ABHH-2			ABGE-1 vs ABGE-2			AB-Wild vs ABHH			AB-Wild vs ABGE		
	χ^2	DF	p	χ^2	DF	p	χ^2	DF	p	χ^2	DF	p
<i>EST-1*</i>	5.61	3	.127	12.73	3	.004*	5.93	3	.133	2.52	3	.480
<i>EST-2*</i>	1.29	2	.545	4.15	2	.148	5.05	2	.094	8.54	2	.021
<i>GPI*</i>	5.88	5	.321	5.36	5	.392	8.37	5	.130	10.84	5	.043
<i>LAP*</i>	0.66	3	.861	9.31	3	.017	0.93	3	.817	54.84	3	<.001*
<i>MPI*</i>	2.93	2	.264	4.42	2	.133	1.34	2	.468	17.80	2	.002*
<i>ODH*</i>	0.69	2	.620	1.63	2	.444	3.08	2	.211	6.17	2	.053
<i>PGM*</i>	2.70	3	.492	2.89	3	.447	2.70	3	.465	2.97	3	.409

Sequential Bonferroni tests were used to keep the overall significance threshold at $\alpha = 0.05$ for each group of multiple comparisons (each column).

* Indicates a significant difference at an overall $\alpha = 0.05$.

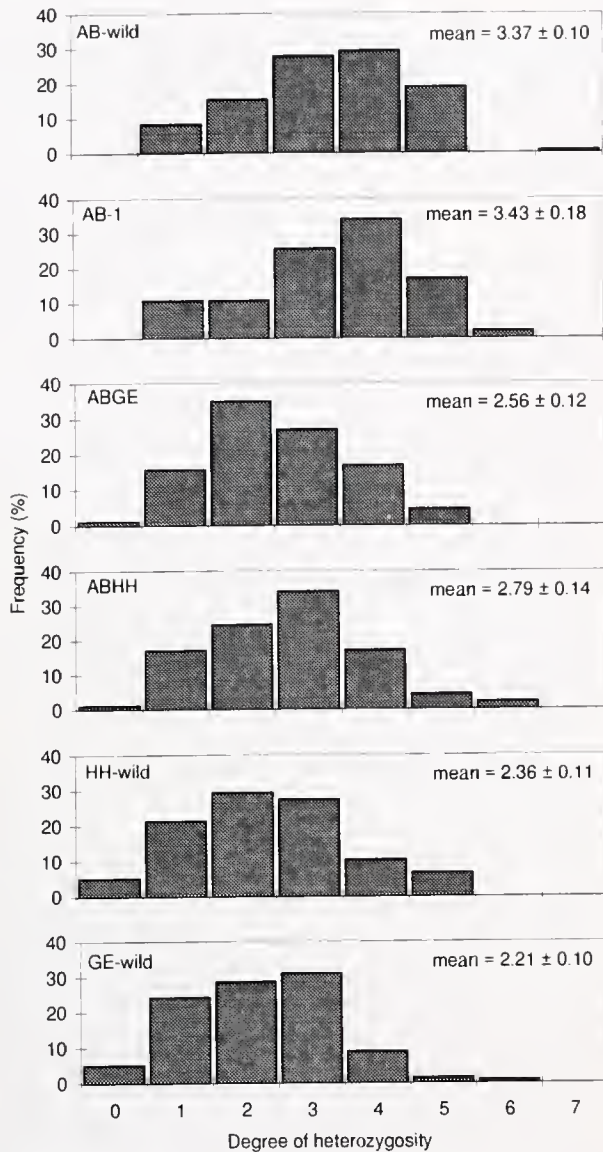


Figure 2. Frequency distributions of the degree of heterozygosity (nb of heterozygous loci per individual) of wild mussels (mussel beds + spat) from the three lagoons (AB-wild, GE-wild, and HH-wild), from mussels left undisturbed on collectors for 1 y in Amherst Basin (AB-1) and from suspension-cultured mussels transferred as spat from Amherst Basin to growout sites in the House Harbour (ABHH) and the Great Entry (ABGE) lagoons for 1 and 2 y combined. Mean number (\pm SE) of heterozygous loci are presented for each group.

result 1995) do not result from different mixtures of *M. edulis* and *M. trossulus*.

We have not detected large differences in allelic frequencies of *M. edulis* from the three studied lagoons which suggests that these mussels belong to a panmictic population. Further, heterogeneity in allelic frequencies seems to be higher within rather than among the lagoons. This is most likely due to the small sample size from some wild beds. For example, the allelic frequencies of HH-c mussels at the *MPI** locus were significantly different from the other mussel beds (Table 4) due to the presence of only two homozygotes for the allele *MPI**-C which is rather rare in these beds (Table 2).

TABLE 6.

F_{ST} indices for each locus and estimated values of the number of migrants per generation ($N_e m$) for wild mussels (mussel beds and spat) in the three lagoons.

Locus	F_{ST}	$N_e m$
<i>EST-1</i>	0.007	36
<i>EST-2</i>	0.003	83
<i>GPI</i>	0.004	62
<i>MPI</i>	0.002	125
<i>ODH</i>	0.001	250
<i>LAP</i>	0.001	250
<i>PGM</i>	0.001	250
Mean over all loci	0.003	83

Homogeneity in allelic frequencies is not surprising given the importance of gene flow that seems to take place among the lagoons. Indeed, an overall $N_e m$ value of 83 is considered very high and largely sufficient to prevent differentiation of populations due to genetic drift (Slatkin 1981). The low values of genetic distances are in agreement with this observation and also indicate low differentiation among the mussels of the different lagoons. This high level of gene flow possibly results from the interaction between the pelagic life of mussel larvae, the water circulation patterns, the topography (small size and shallow depth) of the lagoons, and the prevalence of winds. The pelagic life of mussels typically lasts 2–4 weeks in the Magdalen Islands (Roussy and Myrand 1997) but the duration of this stage could be as long as 6 months for *M. edulis* (Lane et al. 1985). This may ensure a wide dispersal and substantial mixing of different populations (Gosling 1992b). The capacity of byssus drifting by young post-larvae (Lane et al. 1985) may also increase population mixing. Water circulation is rather uniform into each of the three lagoons as a result of their simple topography, and some water flow is observed between the House Harbour and the Great Entry lagoons (Koutitonski and Booth 1996). Maximum depth varies from 3 m in Amherst Basin to 7 m in the House Harbour and the Great Entry lagoons, and the prevalence of winds keeps the water column well mixed (Myrand 1991, Roy et al. 1991).

Wild mussels from the House Harbour and the Great Entry lagoons exhibit an important heterozygote deficiency which is not detected in Amherst Basin. The mean heterozygosity at the 7 loci was 0.32 for wild mussels from the Great Entry lagoon and 0.34 for those from the House Harbour lagoon compared with 0.50 for mussels from Amherst Basin. The mean heterozygosity of mussels from Amherst Basin was in agreement with Hardy-Weinberg equilibrium and corresponds to values obtained from natural populations of adult mussels (Koehn and Gaffney 1984, Diehl and Koehn 1985, Gaffney 1990) and mussels reared in the laboratory (Beaumont et al. 1983). This difference in heterozygosity between Amherst Basin mussels and those from the other two lagoons is surprising given the apparent importance of the gene flow. Thus, we can hypothesize that the changes in heterozygosity occurred after settlement. Selection seems to act against heterozygotes in the Great Entry and the House Harbour lagoons since their proportion is much lower. A more detailed study would be needed to examine possible causes for this difference in heterozygosities between wild stocks. The similar deficiencies in heterozygosity of the

TABLE 7.

Matrix of Nei's unbiased genetic distances calculated for wild mussels (mussel beds and spat) and for suspension-cultured mussels transferred as spat from Amherst Basin to growout sites in the Great Entry and the House Harbour lagoons.

Groups of Mussels	GE-Wild	HH-Wild	AB-Wild	ABGE	ABHH	AB-1
Wild mussels from the Great Entry lagoon (GE-Wild)	—	0.000	0.003	0.035	0.000	0.006
Wild mussels from the House Harbour lagoon (HH-Wild)		—	0.003	0.032	0.002	0.000
Wild mussels from Amherst Basin (AB-Wild)			—	0.026	0.002	0.000
Cultured mussels in the Great Entry lagoon (ABGE)				—	0.034	0.027
Cultured mussels in the House Harbour lagoon (ABHH)					—	0.009
Mussels left on collectors for 1 y in Amherst Basin (AB-1)						—

Suspension-cultured mussels in either the Great Entry or the House Harbour lagoons for 1 and 2 y were pooled together.

House Harbour and the Great Entry mussels compared with mussels from Amherst Basin support the previous suggestion that differences in stocks' susceptibility to summer mortalities in the Magdalen Islands is related to genetic factors since only the latter stock shows a high resistance (Myrand and Gaudreault 1995).

Our results from mussels in Amherst Basin differ from other studies showing an heterozygote deficiency in wild spat (Gosling and Wilkins 1985, Gosling and McGrath 1990) and in laboratory cultures of juvenile mussels (Beaumont et al. 1983, Hvilsom and Theisen 1984, Mallet et al. 1985, Beaumont et al. 1988, 1989, 1990). In these studies, spat was analyzed soon after settlement, i.e., within 2–4 wk, compared with 3–4 mo in the present study. If spat from Amherst Basin also had deficiency in heterozygotes at presettlement stage, it could have been compensated by selective mortality of more homozygous individuals during this 3–4 mo period. Heterozygote deficits seem to be widespread in natural populations (Raymond et al. 1997) but tend to diminish with increasing age (Koehn et al. 1973, 1976, Diehl and Koehn 1985). Further, Zouros and Foltz (1987) proposed a model where heterozygote inferiority (underdominance) at the presettlement stage is compensated by heterozygote superiority (overdominance) at post-settlement stage.

Among the most interesting results of this study are the decreases in heterozygosity that we observed in suspension-cultured mussels which were transferred as spat from Amherst Basin ($H_o = 0.50$) to growout sites in the Great Entry ($H_o = 0.36$) and the House Harbour ($H_o = 0.38$) lagoons. The similar heterozygosity values measured in mussels transferred for 1 and 2 ys in both lagoons (Table 3) suggest that spat from Amherst Basin suffered substantial losses of heterozygotes during its first year in suspension culture. It can be hypothesized that such losses of heterozygous individuals may be caused by the environmental conditions prevailing at the growout sites. However, these conditions appear to be quite comparable to those observed in Amherst Basin (Myrand and Gaudreault 1995). Further, this hypothesis would be difficult to reconcile with several studies reporting that stressful conditions usually emphasize heterosis (Rodhouse and Gaffney 1984, Gentili and Beaumont 1989, Hawkins et al. 1989, Koehn and Bayne 1989, Scott and Koehn 1990, Borsa et al. 1992). Another explanation may lie in the suspension-culture practices adopted in the Magdalen Islands. We hypothesize that after sleeving the more heterozygous spat tend to go out of the mesh sleeves quicker than the more homozygous spat to get a better access to the surrounding environment (for details on mussel culture see Mallet and Myrand 1995). As byssal attachment is probably too weak to keep them firmly attached to the sleeves, they are more prone to fall-off caused by turbulence in the shallow lagoons resulting from wind storms. Gentili and Beaumont (1989) already suggested that more

heterozygous mussels are more active and thus actively maintain their position at the outside of clumps on sea bottom. Mussels left undisturbed on collectors in Amherst Basin during 1 y showed no such decrease in heterozygosity and thus were similar to wild mussels (including spat). Therefore, artificial collectors seem to have no effects on genotypic structure of mussels, at least in Amherst Basin. Given the inverse relationship observed between heterozygosity and metabolic needs for different stocks (Tremblay et al. 1998c), it seems that a substantial part of the individuals with an energetic advantage (more heterozygous) are lost after spat collectors are harvested for sleeving.

It is surprising that the allelic frequencies of suspension-cultured mussels differed between the two growout sites given their common origin and their similar degree of heterozygosity. The selectivity against heterozygotes in suspension-culture is possibly not related to specific loci as the proposed mechanism relies on "passive" losses due to fall-off. As two different mussel growers provided these mussels (one in each lagoon), the observed differences may possibly be related to variable culture practices such as sleeving periods and stocking densities. As Fréchette et al. (1996) observed higher self-thinning (fall-off) on sleeves with higher stocking densities, it is plausible that variable self-thinning could create the differences observed in the present study.

This study provides useful basic information about genetic characteristics of blue mussels in the Magdalen Islands. Wild mussels from the three lagoons did not differ according to their relative composition of *M. trossulus* and *M. edulis* and the latter is largely prominent. Allelic frequencies were similar among the lagoons because of the high gene flow and the apparent absence of selection on particular alleles. However, heterozygosity in mussels from Amherst Basin was higher than in those from the other lagoons. Further, mussels transferred as spat from Amherst Basin to growout sites in the House Harbour and the Great Entry lagoons showed important losses in heterozygotes within the first year. This study raises serious concerns about the possible impacts of aquaculture practices on some genetic characteristics of suspension-cultured mussels. The results of this study may have major consequences on mussel culture practices in the Magdalen Islands because of the necessity to preserve the high quality (resistance to summer mortality) of these Amherst Basin mussels all along the production cycle and to protect its genetic integrity. A study is currently under way to examine this question.

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MOLECULAR IDENTIFICATION OF FOUR SPECIES OF MUSSELS FROM SOUTHERN CHILE BY PCR-BASED NUCLEAR MARKERS: THE POTENTIAL USE IN STUDIES INVOLVING PLANKTONIC SURVEYS

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ABSTRACT PCR amplification of two nuclear DNA fragments (*ITS* and *GLU-5*) and subsequent restriction fragment polymorphism (RFLP) were applied to mantle tissue samples from four species of Chilean mussels, *Mytilus edulis chilensis*, *Choromytilus chorus*, *Aulacomya ater*, and *Perumytilus purpuratus*, and to individual D-shape larvae of *Mytilus edulis* and *M. trossulus* from Newfoundland, in order to look for specific banding patterns for each of the Chilean species. Both nuclear DNA-based markers produced species-specific banding patterns and may therefore be able to differentiate larvae from the four Chilean mussels during studies that rely on plankton surveys.

KEY WORDS: Polymerase chain reaction, species identification, mussels, larvae, Chile

INTRODUCTION

Most marine bivalves have a prolonged, mobile, planktonic larval stage, giving the potential for wide dispersal of larvae (Cradock et al. 1995). Studies carried out to provide an insight into the relationship between benthic mussel communities and planktonic distribution of mussel larvae are based on the use of detailed morphological analyses of plankton samples (Ramorino and Campos 1983, Pulfrish 1997). When these investigations are carried out in areas where more than one mussel species are sympatrically or parapatrically present, some difficulties regarding the identification of larvae are often encountered (Cragg 1996, Olsen 1991), especially in temperate waters where the reproduction is highly seasonal determined (Bayne 1976) and most invertebrate species have overlapping spawning times (Olive 1992, Michin 1993). Moreover, the size and shape of prodissoconch II shells of larval bivalves vary considerable (Loosanoff and Davis 1963, Bayne 1965, Pulfrish 1997), making the use of identification guides more difficult. In southern Chile, mussels species from four genera—*Mytilus*, *Choromytilus*, *Aulacomya*, and *Perumytilus*—are often found sharing the same ecosystem (Solís and Lozada 1971, Lozada et al. 1974, Varela and Valenzuela 1983, Winter et al. 1984, Simpfendorfer et al. 1995) and therefore difficulties are expected when analyzing plankton samples for mussel larvae identification. Molecular genetics markers have become indispensable tools in the study of natural populations of marine organisms (Avise 1987). The polymerase chain reaction (PCR) (Mullis and Faloona 1987, Buffery 1993) has the potential to afford a sufficiently sensitive method to be applied to invertebrate larvae (Olson et al. 1991) such as *Mytilus edulis* larvae (Côté-Real et al. 1994, Sutherland et al. 1998) and *M. edulis* and *Mytilus trossulus* larvae at very early stages (<48 h) (Toro unpublished data). This technique, using specific genetic markers, could be very useful as an aid to the studies involving planktonic surveys of bivalve larvae.

MATERIAL AND METHODS

Chilean mussels *Mytilus edulis chilensis* (Soot-Ryen, 1955), *Aulacomya ater* (Molina, 1782), *Choromytilus chorus* (Molina,

1782), and *Perumytilus purpuratus* (Lamarck) were collected at Corral Bay and at the location of Chaihuín, X Región, southern Chile. Each mussel ($n = 20$) was dissected and a small piece of the mantle border was fixed in 95% ethanol. Approximately 50–100 mg of each fixed mantle-edge tissue was coarsely chopped and digested in 500 μ l of lysis buffer (50 mM Tris-HCl (pH 8.0); 1.0% SDS; 25 mM EDTA) with 200 μ g proteinase K at 37°C overnight. The solution was then extracted once with 500 μ l of an equal volume of phenol-chloroform-isoamyl alcohol (24:24:1) followed by ethanol precipitation. The extracted DNA was resuspended in 200 μ l of ultra-pure sterile distilled water. A PCR-based nuclear species marker developed by Heath et al. (1995), based on the internal transcribed spacer (ITS) regions between the 18S and 28S nuclear rDNA coding regions was applied in the present study. The primers used were ITS 1 5'-TTTCCGTAGGTGAACCTG-3' and ITS2 5'-CTCGTCTGATCTGAGGTCG-3', with an expected PCR gene fragment size of 1,250 bp. Standard PCR amplifications were carried out in 25- μ l reaction mixtures containing DNA template, 0.2 mM each of the four deoxyribonucleotide triphosphates (dNTPs), 2.0 mM $MgCl_2$, primers at 0.4 mM, 1 unit of Taq DNA polymerase (Promega), the manufacturer-supplied PCR buffer, and sterile distilled water. The reaction mixtures were overlaid with a drop of mineral oil to prevent evaporation, and were then placed in a programmable thermocycler (MJ Research, Inc). The thermal cycler protocol consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 20 sec, 50°C for 20 sec, and 72°C for 2 min. Following the DNA amplification, a restriction analysis was applied to each PCR product obtained. An amount of 5 μ l of each amplified PCR-products was digested for 12 h at 37°C with 0.5 U of the restriction enzyme HhaI in a total volume of 15 μ l, including 3 μ l of buffer supplied by the manufacturer (Pharmacia) and 6.5 μ l of ultra-pure distilled water. The digested products were electrophoresed for 30 min at 112 V on 3% agarose gels in 0.5 \times TBE (Tris-borate-EDTA) buffer. The restriction fragment length polymorphisms (RFLPs) were visualized by placing the gels in a solution of ethidium bromide and photographed under ultraviolet illumination. A second nuclear-DNA marker developed by Rawson et al., (1996), targets the gene encoding the mussel polyphenolic adhesive protein. This protein is produced by the endocrine gland in the foot of a mussel and is used for its attachment to the substrate. The primers used were JH-5

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5'-GTAGGAACAAAGCATGAACCA-3' and the reverse primer JH54 5'-GGGGGGATAAGTTTCTTAGG-3'. PCR amplifications were carried out in 25- μ l reaction mixtures containing approximately 50 ng of DNA template, 2.5 nmol of dNTPs, 2.0 mM MgCl₂, 50 pmol of each primer, and 1 U of Taq DNA polymerase (Promega), the manufacturer-supplied PCR buffer, and sterile distilled water. The reaction mixtures were overlaid with a drop of mineral oil to prevent evaporation, and were then placed in a programmable thermocycler. The thermal cycling protocol consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 20 sec, 53°C for 20 sec and 72°C for 45 sec. PCR products were then directly resolved on 3% agarose gels stained with ethidium bromide and scored for species using Polaroid photos taken under UV light.

Larvae from *M. edulis* and *M. trossulus* were obtained by several plankton tows (20 μ m mesh) at Bellevue, and Chance Cove, Newfoundland during September 1996. Also, larvae from pure *M. edulis* and *M. trossulus* were obtained by rearing larvae from artificial laboratory crosses of the two species (Scarpa et al. 1994, Toro and Sastre 1995, Toro and Paredes 1996). Total DNA extraction from 200 individual larvae from each species at each location and 60 from each pure larval culture was carried out. The procedure was similar to that described for the mantle tissue, except that larvae were previously measured and isolated under a stereo microscope (at 40 \times magnification) using a Pasteur pipette. The individual larva was then placed in a 1.5 ml Eppendorf tube and washed twice in 0.5 ml distilled water before DNA extraction. Individual mussels were scored for genotype at each marker locus on the basis of diagnostic RFLPs. All samples were analyzed at Memorial University of Newfoundland.

RESULTS

The restriction digestion of the 1,250 bp ITS PCR amplified product with HhaI produced four specific RFLPs (Fig. 1). In *M. edulis chilensis*, the 1,250 bp fragment was cut into two 450 bp fragments and two 180 bp fragments. In *Ch. chorus*, the ITS-PCR product was cut into two 344 bp fragments, two 160 bp fragments, and several <100 bp fragments. In *A. ater*, a 440 bp fragment, 280 bp fragment, 160 bp fragment, and several <100 bp fragments

were produced. In *P. purpuratus*, a 300 bp fragment, 260 bp fragment, 220 bp fragment, 180 bp fragment, and several <134 bp fragments were produced. This assay was able to distinguish all four species of Chilean mussels (Fig. 1). In all *Mytilus edulis* larvae, the banding pattern was similar to *M. edulis chilensis*. In all *Mytilus trossulus* larvae, the ITS-PCR product was cut into two 280 bp fragments, two 180 bp fragments, and several <100 bp fragments (Fig. 1).

The Glu-5 PCR assay produced also species-specific banding patterns, except for *P. purpuratus* (Fig. 2). In *M. edulis chilensis*, three primary bands of 200, 300, and 500 bp were produced. When this assay was applied to *Ch. chorus*, four primary bands of 540, 330, 290, and 200 bp were produced. In *A. ater*, a strong band at 560 bp and two less clear bands at 396 bp and 200 bp were detected. In *P. purpuratus*, this PCR assay produced several <134 bp bands that were not clearly resolved in the 3% agarose gels. In *Mytilus edulis* larvae, this PCR assay produced a single 350 bp band and in *M. trossulus* larvae a single primary band of 240 bp was produced (Fig. 2).

The results from the two nuclear DNA-based markers were consistent among individuals within species, between adults and larvae and also within species samples from different geographic regions.

DISCUSSION

Both DNA-based markers produced species-specific banding patterns and may therefore be able to differentiate larvae from the four Chilean mussels during studies that rely on plankton surveys. PCR-based assays of genetic markers have become a valuable tool in ecological studies: they are more sensitive than allozyme techniques, and can be applied in minute quantities of material such as bivalve planktonic larvae, even before the deposition of the final larval shell, which is a requisite for a more accurate species identification based on shell shape (Pulfrish 1997).

The mussel larvae is associated with a free-swimming planktonic stage; the larvae spend several weeks feeding in the water column and are capable of dispersing hundreds of kilometers (Bayne 1965, Chanley and Andrews 1971, Craddock et al. 1995). This has limited studies of ecology and recruitment processes in these invertebrates (Butman 1987, Olsen 1991, Pawlik 1992, Rodriguez et al. 1993, but see Hunt and Scheibling 1998). Moreover,

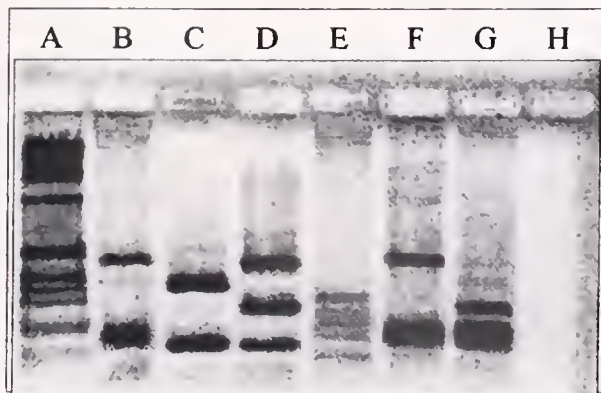


Figure 1. Photo-negative of ethidium bromide-stained agarose gel transilluminated with ultraviolet light showing the RFLP patterns produced by the digested PCR product of ITS from the four species of Chilean marine mussels; *Mytilus edulis chilensis* (lane B), *Choromytilus chorus* (lane C), *Aulacomya ater* (lane D), *Perumytilus purpuratus* (lane E), and from the D-shape larvae from *Mytilus edulis* (lane F), *Mytilus trossulus* (lane G). Line A: molecular weight marker (Gibco BRL 1 kb ladder) and line H: negative PCR control.

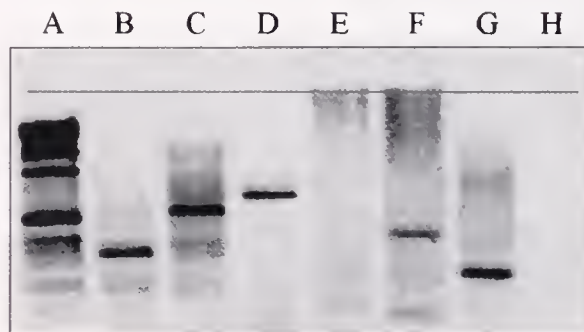


Figure 2. Photo-negative of ethidium bromide stained agarose gel transilluminated with ultraviolet light showing the RFLP patterns produced by the PCR-product of GLU-5 from the four species of Chilean marine mussels; *Mytilus edulis chilensis* (lane B), *Choromytilus chorus* (lane C), *Aulacomya ater* (lane D), *Perumytilus purpuratus* (lane E) and from the D-shape larvae from *Mytilus edulis* (lane F), *Mytilus trossulus* (lane G). Line A: molecular weight marker (Gibco BRL 1 kb ladder) and line H: negative PCR control.

the potential transport of exotic marine species as plankton in ballast water (Carlton and Geller 1993, Geller et al. 1994, Heath et al. 1995) has also been of great concern and this has stressed the need for accurate larval identifications. In the present study, wild mussel larvae <150 μm from plankton tows and D-shape larvae from laboratory crosses, preserved in 95% ethanol, were successfully genotyped using two PCR-based markers. This technique and the use of PCR primers that are highly specific to the target species is therefore potentially very useful in studies concerning the ecology of mussel larvae. Also, population genetic analyses of natural populations, including all stages and age-classes, are now feasible and eventually this could provide more evidence of the importance

of selective and random processes affecting larval dispersal, patterns of settlement, postsettlement transport of juvenile molluscs, and recruitment.

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SELECTIVE FEEDING AND BIODEPOSITION BY ZEBRA MUSSELS AND THEIR RELATION TO CHANGES IN PHYTOPLANKTON COMPOSITION AND SESTON LOAD

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ABSTRACT We examined the ability of zebra mussels (*Dreissena polymorpha*) to preferentially ingest or reject various phytoplankton species and nonliving particles. Our objective was to determine if preferential ingestion by zebra mussels could explain the changes observed in the Hudson River since their invasion: (1) decline of cyanobacteria, *Microcystis* in particular, (2) rise to dominance by diatoms, and (3) very small change in total seston load. We found that zebra mussels are capable of efficiently sorting and rejecting particles. Not only were clearance rates higher when the cyanobacterium *Microcystis* was present in suspension, but *Microcystis* was preferentially ingested over almost all other particle types tested. Diatoms were generally rejected as diffuse pseudofeces which were easily resuspended, even in still water. The rejection of cattail (*Typha*) detritus by zebra mussels corresponds to the rejection by oysters (*Crassostrea virginica*) of cord grass (*Spartina*) detritus particles (Ward et al. 1998). Pseudofeces of clay or detritus particle types were also very diffuse. In a few cases, however, clay or detritus particles, rather than phytoplankton cells, were preferentially ingested by zebra mussels. The interaction of selective feeding by zebra mussels with resuspension of diffuse biodeposits by tidal mixing may explain the differential decline of phytoplankton groups and nonliving particles in the Hudson River.

KEY WORDS: Zebra mussels, particle selection, clearance rates

INTRODUCTION

Suspension-feeding organisms, such as bivalves, can influence the function of ecosystems to a great extent. In dense populations, bivalves can dominate total ecosystem metabolism (Murphy and Kremer 1985, Boucher-Rodoni and Boucher 1990, Dame et al. 1992), nutrient cycling (Jordon and Valiela 1982, Dame et al. 1991, Asmus et al. 1995), and grazing of primary producers (Cloern 1982, Officer et al. 1982). Grazing of primary producers moves energy through ecosystems by coupling pelagic and benthic processes; organic materials are removed from suspension and are deposited on the bottom as feces or pseudofeces, or excreted back to the water column (Dame and Patten 1981, Newell and Field 1983, Smaal and Prins 1993). In many estuarine and coastal systems, bivalves effectively control phytoplankton biomass, harvesting up to 100% of the phytoplankton primary production (Carlson et al. 1984, Asmus et al. 1990, Gerritsen et al. 1994).

The introduction and spread of the zebra mussel (*Dreissena polymorpha*) (Pallas) has added a previously absent guild of organisms to North American freshwater ecosystems. Though native bivalves and other organisms certainly contribute to phytoplankton grazing (Strayer et al. 1994, Cahoon and Owen 1996, Caraco et al. 1997), zebra mussels are fouling organisms that can attain extremely high densities and have the capacity to filter large quantities of water (Reeders et al. 1989, Roditi et al. 1996). As a result of their great clearance rates, phytoplankton biomass has decreased by over 60% in many systems in which zebra mussels have become established (Holland 1993, Leach 1993, Fahnenstiel et al. 1995). Phytoplankton biomass in the Hudson River, New York, has dropped by 90% since zebra mussels invaded in 1991 (Caraco et al. 1997). In some systems, phytoplankton composition has also changed since invasion (Heath et al. 1995, Vanderploeg et al. 1996). The Hudson River phytoplankton community has shifted from prevalence of cyanobacteria to diatoms (Smith et al. in press).

Despite the massive reduction in phytoplankton biomass in the Hudson River, water transparency has increased by only 12%, owing to the persistence of nonliving particles (Caraco et al. 1997, Strayer et al. in press). This is in contrast to other systems in which transparency has increased by 33–100% (Holland 1993, MacIsaac and Rocha 1995).

Studies on marine bivalves have demonstrated their ability to sort particles based on size (Vahl 1972, Stenton-Dozey and Brown 1992, Defosse and Hawkins 1997) and quality (MacDonald and Ward 1994, Arifin and Bendell-Young 1997, Ward et al. 1997). However, the capacity to sort and preferentially ingest particles varies among bivalve species (Möhlenberg and Riisgård 1978, Prins et al. 1991, Ward et al. 1998). It is evident that drops in phytoplankton biomass are the result of zebra mussel filtration, but it is less clear to what extent zebra mussels are directly responsible for changes in phytoplankton community composition. In this study we examined preferential ingestion by zebra mussels of various Hudson River phytoplankton species and nonliving particles. Our objective was to determine if differential ingestion and rejection by zebra mussels could explain the observed changes in the Hudson River phytoplankton community and the lack of change in turbidity. In addition, we examined the effect of suspension complexity on sorting, and compared selection of phytoplankton species with information on assimilation efficiencies. This is the first study to determine zebra mussel particle preferences by directly examining pseudofecal composition by means of flow cytometry.

MATERIALS AND METHODS

Mussels

Specimens of *Dreissena polymorpha* were collected from the Hudson River at Tivoli, New York, or from the Huron River, Ann Arbor, Michigan. Mussels were maintained in 40 L aquaria at

16°C and fed a daily ration of cultured phytoplankton plus a mixture of preserved diatoms (Diet C, Coast Seafoods, Co., Quilcene, Wash). Partial water changes (ca. 20%) were performed on alternating days; freshwater was prepared according to Sprung (1987).

Particles

Phytoplankton cultures were obtained from the University of Texas Culture Collection and grown in a modified f/2 media (Guillard and Hargraves 1993); working stock solutions were added to distilled water, rather than seawater, resulting in 0 ppt salinity. Cultures were grown at room temperature, under a 16:8 h light and dark regime. Species of phytoplankton that are typically found in the Hudson River were cultured for use in experiments: *Cyclotella meneghiniana* (LB 2455; barrel-shaped, 18 X 6 µm), freshwater-acclimated *Thalassiosira* sp. (LB 2054; barrel-shaped, 15 X 13 µm) (Bacillariophyceae), *Micractinium* sp. (LB 2614; spherical, 6 µm), *Crucigenia tetrapedia* (63; disk-shaped, 5 X 11 µm), *Scenedesmus quadricauda* (LB 614; four cells stacked, total 25 X 10 µm) (Chlorophyceae), and *Microcystis aeruginosa* (LB 2386; spherical, 4 µm) (Cyanophyceae). Cells were measured using an ocular micrometer.

Nonliving particles of detritus and clay were also used in the experiments. Dead cattail (*Typha* sp.) leaves from the previous growing season were collected from a marsh on the Hudson River for use as detrital material. Leaves were washed of debris, and processed in a blender with distilled water for 5 min. The resulting suspension was sieved through a nylon screen to include particles less than 20 µm; 90% of the particles were ≤3.5 µm, as measured by a Coulter Multisizer. Clay suspensions were produced by adding kaolin (hydrated aluminum silicate, Fisher Scientific, Co., Pittsburgh, PA) to distilled water and agitating vigorously. All clay particles were ≤20 µm and 90% of the particles were ≤2.5 µm. Both *Typha* detritus and clay suspensions were made 1 day before use in experiments and were refrigerated overnight.

Particle Selection

A series of particle selectivity experiments was performed. Zebra mussels were scrubbed and allowed to purge themselves for 24 h before experiments. Particle suspensions were prepared by diluting phytoplankton cultures and/or nonliving particle stock solutions with filtered (0.45 µm) Hudson River freshwater to total concentrations of 10⁵ particles mL⁻¹. Combinations of two or three particle types were provided in nearly equal proportions. Individual mussels were placed in beakers in 200 mL of the particle suspension. Ten experimental beakers and two or three control beakers, without mussels, were run concurrently. To keep particles homogeneously in suspension, beakers were gently aerated throughout the measurements. The behavior of the mussels, whether open or closed, was carefully monitored. Water samples of 1 mL each were taken at the beginning of the experiments and after 30–90 min. Particle concentrations did not decline below 65% of the starting value. Biodeposits were removed from the beakers as they were produced by the mussels; feces were discarded and pseudofeces were collected for analysis. Following experiments, the mussels were measured, the tissues were removed from the shells, and the tissue dry mass was determined by oven drying at 60°C for 24 h.

The abundance of particle types in water samples and pseudofeces was determined using a FACScan portable flow cytometer (Becton Dickinson, San Jose, CA) equipped with a 15 mW, 488 nm

argon laser. Samples of both water and pseudofeces were agitated vigorously before analysis to disrupt any aggregations. Phytoplankton cells were differentiated by chlorophyll fluorescence (>650 nm) and phycoerythrin fluorescence (560–590 nm) emissions, forward scatter (a measure of size), and 90° side scatter. Nonliving particles were differentiated from phytoplankton cells based on their lack of pigmentation, as well as forward and side scatter. The volume of sample analyzed was determined gravimetrically.

The proportions of particle types in the samples were determined from the flow cytometry data. To examine the degree of acceptance or rejection of particle types, we calculated a modified electivity index (EI) as follows:

$$EI = -[(P - S) / ((P + S) - (2 * P * S))]$$

where P is the particle ratio in the pseudofeces and S is the particle ratio in the suspension (Jacobs 1974, Bayne et al. 1977). Electivity index can range from -1.0 to 1.0. A positive EI for a given particle type indicates preferential ingestion (depletion of the particle type in the pseudofeces compared with the suspension), and a negative EI indicates rejection (enrichment of the particle type in the pseudofeces compared with the suspension). Electivity indices were compared with zero using a one-sample, two-tailed, nonparametric Wilcoxon signed-rank test. These analyses test the null hypothesis that electivity of a particular particle type is equal to zero (no sorting).

To better illustrate the efficiency of particle selection, sorting efficiency (Iglesias et al. 1992, MacDonald and Ward 1994) was calculated:

$$SE = 1 - (P/S)$$

This index, which ranges from 0 to 1, represents the percentage enrichment or depletion of a particle type in the pseudofeces compared with the suspension.

Clearance Rates

Particle depletion data from the above selectivity experiments were used to calculate clearance rates (mL h⁻¹). Some clearance rates were determined separately from the selectivity experiments. These experiments were conducted in the same manner as the selectivity experiments except as follows. Fifteen experimental beakers and three control beakers were run concurrently. Feces and pseudofeces were removed from the beakers as they were produced by the mussels; both were discarded. The abundance of particles in the water samples was determined using a Coulter Multisizer II, equipped with a 75 µm aperture tube, and set to draw 500 µL. Samples were diluted with electrolyte solution and gently agitated. Counts were corrected for dilution and background count.

Particle depletion data from either the flow cytometer or Coulter Multisizer was used to calculate clearance rates according to Coughlan (1969). Clearance rates were corrected for particle abundance changes in the controls and for the time that each mussel was open. Clearance rates were standardized to a 15 mg dry tissue mass (corresponding to a mussel of approximately 20 mm in length) using the allometric exponent for bivalves of 0.88 (Kryger and Riisgård 1988).

Analyses of variance were performed for groups of experiments with at least one common particle type to test the null hypotheses that there were no effects of particle combination on clearance rates. If a null hypothesis was rejected, Dunnett's multiple comparison test was used to identify specific two-particle

clearance rates that differed from the clearance rate of the common particle alone. Statistical analyses were conducted using JMP version 3.1.6 software (SAS Institute Inc. 1994). A significance level of 0.05 was used.

RESULTS

Particle Selection

At the end of all experiments, proportions of provided particles were not significantly different in the experimental beakers with zebra mussels than they were in control beakers. This indicates that zebra mussels removed different particle types and particle sizes from suspension with equal efficiency. Analyses of pseudofeces, however, showed that zebra mussels sort particles for rejection or ingestion once they have entered the mantle cavity, on the gills and/or labial palps. Most particle combinations tested resulted in significant EIs (Fig. 1). Though there was a tendency for smaller particles to be accepted for ingestion over larger particles, this was not always the case (Fig. 1).

The cyanobacterium, *Microcystis*, was preferentially ingested over nearly all other particles (Fig. 1C). Sorting efficiencies indicate that pseudofeces were depleted of *Microcystis* by up to 67%, compared with the suspension. There was no sorting between clay and *Microcystis* (Fig. 1C).

Zebra mussels generally rejected species of green phytoplankton with larger cell sizes. *Scenedesmus* was rejected in favor of

other particles (Fig. 1C, 1F). *Crucigenia* was rejected in favor of *Microcystis* and nonliving particles (Fig. 1D), although there was no sorting between *Crucigenia* and a smaller green, *Micractinium*, or a diatom, *Thalassiosira* (Fig. 1D). *Micractinium* was rejected in favor of *Microcystis* (Fig. 1C) but was preferentially ingested over a large diatom, *Cyclotella* (Fig. 1F).

Diatoms were either rejected or there was no sorting between them and other particles (Fig. 1E, 1F). Only *Scenedesmus* was rejected in favor of a diatom (Fig. 1F). *Thalassiosira* was rejected in favor of *Microcystis* (Fig. 1E), and there was no sorting between *Thalassiosira* and *Crucigenia* (Fig. 1D), or the larger diatom, *Cyclotella* (Fig. 1E). *Cyclotella* was rejected in favor of *Microcystis* and *Micractinium*, and the degree of rejection relative to *Typha* detritus was nearly significant as well ($p = .062$) (Fig. 1F). There was no sorting between *Cyclotella* and clay particles (Fig. 1F).

Phytoplankton cells were not always preferentially ingested over nonliving particles (Fig. 1A, 1B). Clay particles were preferentially ingested over *Crucigenia*, and there was no sorting between clay and *Microcystis* or *Cyclotella* (Fig. 1A). *Typha* detritus was rejected in favor of *Microcystis*, but was preferentially ingested over *Crucigenia*, and nearly significantly accepted over *Cyclotella* (Fig. 1B).

The sign (either positive or negative) and magnitude of the electivity index for a given particle type depended on the complexity of the suspension. For example, in paired suspensions, *Microcystis* was preferentially ingested over both diatoms *Thalassiosira* and *Cyclotella* (Fig. 1C), and there was no sorting between the two diatoms when paired with each other (Fig. 1E, 1F). In suspensions of these three particle types together, however, the EI for *Cyclotella* shifted to positive (acceptance) (Fig. 2A). Similar shifts from significant rejection to acceptance (although not significant) occurred for *Micractinium* (Fig. 2B) and for *Crucigenia* (Fig. 2C) in suspensions with *Microcystis* and *Scenedesmus*.

During experiments, we also observed the consistency and integrity of pseudofeces. Pseudofeces consisting mainly of large green cells were ejected as compact balls that remained intact for long periods of time, min to h. Pseudofeces consisting mainly of diatoms or clay particles were ejected as diffuse, nondiscrete masses from the inhalant siphon, or were ejected as a particulate cloud from the byssal gape. These types of pseudofeces dispersed into the water column within a few sec, even in still water. Cultures were successfully started from the resuspended cells.

Acceptance or rejection of:

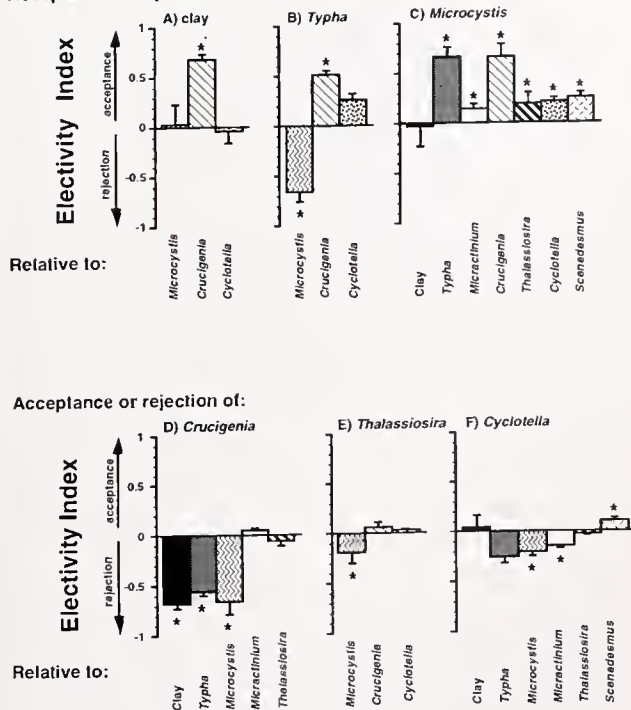


Figure 1. *Dreissena polymorpha*. Electivity indices for (A) clay, (B) *Typha* detritus, (C) *Microcystis*, (D) *Crucigenia*, (E) *Thalassiosira*, and (F) *Cyclotella* relative to particle types listed across the bottom. Particle types are listed in order of ascending size. A positive EI indicates selection of the particle type in the heading. A negative EI indicates a rejection of the particle type in the heading (and therefore selection of the particle type listed on the bottom). * Indices significantly different than zero ($p < .05$). (Means \pm SE, $n = 10$).

Clearance Rates

The clearance rate of *Microcystis* alone was greater than that of the other single-particle type suspensions (Fig. 3). Total clearance rates of suspensions with different particle types combined also differed (Fig. 3A–F), even though relative clearance rates of the individual particle types within a given suspension did not (as shown by no significant change in suspended particle proportions). Total clearance rates appeared to be unrelated to the desirability of the individual particle types in suspension. For example, although *Thalassiosira*, and especially *Typha*, were rejected when paired with *Microcystis* (Fig. 1C), clearance rates for suspensions of these particles with *Microcystis* were not less than that of *Microcystis* alone (Fig. 3C). Clearance rates of combinations of particle types were generally equal to or less than that of the common particle types alone (Fig. 3A–D). Diatoms were an exception to this, however. Addition of *Microcystis* to suspensions of *Thalassiosira*, and addition of *Micractinium* or clay to suspensions of *Cyclotella*,

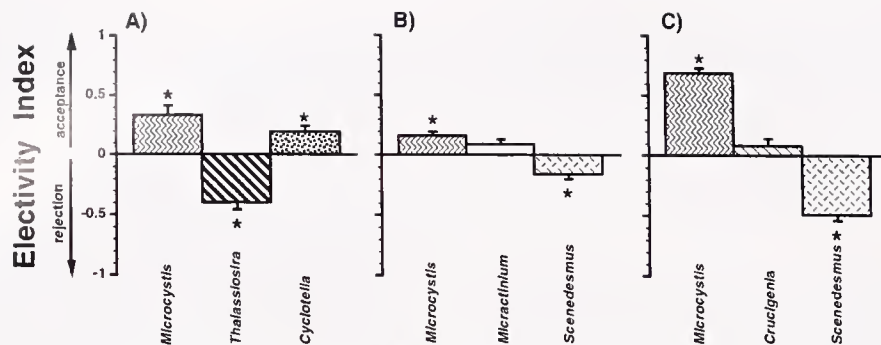


Figure 2. *Dreissena polymorpha*. Electivity indices for individual particle types in suspensions of three particle types. A positive EI indicates selection of the particle type, a negative EI indicates a rejection of the particle type. Suspensions of (A) *Microcystis*, *Thalassiosira*, and *Cyclotella*, (B) *Microcystis*, *Microactinium*, and *Scenedesmus*, and (C) *Microcystis*, *Crucigenia*, and *Scenedesmus*. * Indices significantly different than zero ($p < .05$). (Means \pm SE, $n = 10$).

increased clearance rates above that of the diatoms alone (Fig. 3E, 3F).

DISCUSSION

Our study is the first to determine zebra mussel selection of phytoplankton species by directly examining pseudofeces by means of flow cytometry. We found that zebra mussels are capable

of efficiently sorting particles. Our results are consistent with the changes in phytoplankton community composition that have occurred in the Hudson River estuary since invasion by the zebra mussel. The main phenomena in need of explanation were (1) the decline of cyanobacteria, *Microcystis* in particular, (2) the rise to dominance by diatoms, and (3) the very small change in total seston load, which is dominated by clay particles. The interaction of selective feeding by zebra mussels with resuspension of diffuse biodeposits by river mixing, due in part to tidal forces, may explain the differential decline in phytoplankton groups that has occurred in the Hudson River.

Before invasion of the Hudson River by zebra mussels, colonial and single-celled cyanobacteria, especially *Microcystis aeruginosa* and *Microcystis* sp., often reached summer bloom densities of over 10^7 cells L^{-1} (Howells and Weaver 1969, Marshall 1988). Since then, cyanobacteria have nearly disappeared from the river (Smith et al. in press), decreasing from 36 to 4% of the total number of cells (Marshall 1988, Smith et al. in press). Similarly, Noordhuis et al. (1992) documented the absence of cyanobacteria blooms in Dutch ponds following zebra mussel stocking. Consistent with the disappearance of cyanobacteria from the Hudson River and from the Dutch ponds, we found that not only were zebra mussel clearance rates higher when *Microcystis* was present in suspension, but also that *Microcystis* was preferentially ingested over almost all other particle types tested. Our results corroborate those of other workers. Bastviken et al. (1998) indirectly measured selection in short-term microcosm experiments by comparing gross (no resuspension of feces and pseudofeces) and net (feces and pseudofeces resuspended) clearance rates for different phytoplankton. They found that single-celled *Microcystis* is among those phytoplankton cleared most efficiently from Hudson River water by zebra mussels.

In some lake systems, however, zebra mussels are reported to promote *Microcystis* blooms (Vanderploeg et al. 1996) or have no effect on *Microcystis* abundance (Lavrentyev et al. 1995), suggesting that *Microcystis* is not a preferred food. Blooms of cyanobacteria have been observed in Lake Erie, Saginaw Bay, and Oneida Lake since zebra mussels invaded (Health et al. 1995, MacIsaac 1996, Vanderploeg et al. 1996), and microcosm experiments in Saginaw Bay show that zebra mussels have no effect on the abundance of *Microcystis* (Lavrentyev et al. 1995). In these cases, there may be mechanical or chemical inhibition of clearance of *Microcystis* by zebra mussels. For example, *Microcystis* in Saginaw Bay is primarily in the form of large, gelatinous colonies (Lavrentyev

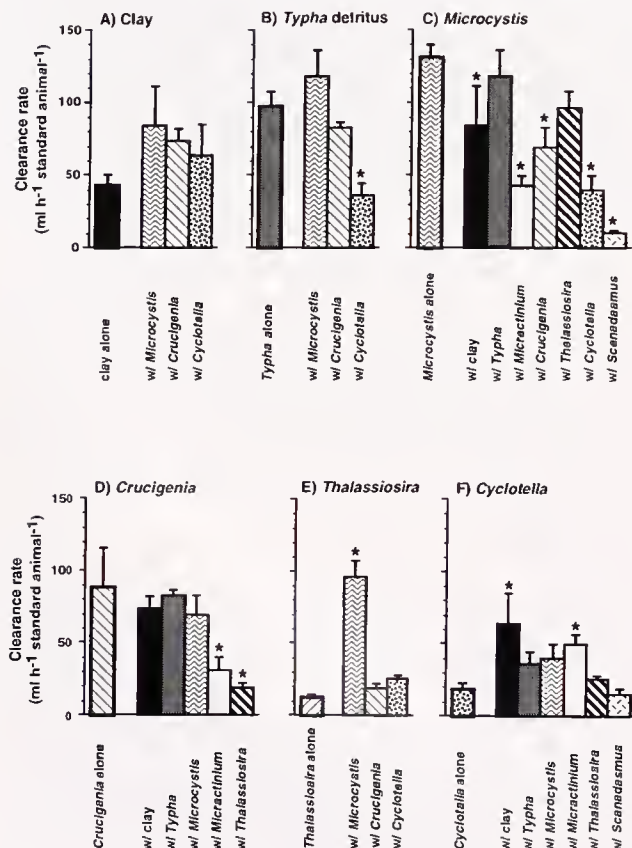


Figure 3. *Dreissena polymorpha*. Clearance rates of single-particle type suspensions and pairs of particle types, standardized to an animal of 15 mg dry tissue mass. Clearance rates of combination suspensions were compared with those of the common particle types alone: (A) clay, (B) *Typha* detritus, (C) *Microcystis*, (D) *Crucigenia*, (E) *Thalassiosira*, and (F) *Cyclotella*. * Clearance rates significantly different than the common particle type alone ($p < .05$). (Means \pm SE, $n = 15$).

et al. 1995) which appear to mechanically disturb filtering in some bivalves (Kamermans 1992, Smaal and Twisk 1997). In addition, some strains of *Microcystis aeruginosa* are toxic to bivalves, zooplankton, and fish, causing mortalities or reduced feeding activity (Birger et al. 1978, Keshavanath et al. 1994, Shaw et al. 1997). However, we have found that clearance rates of a toxic strain of single-celled *Microcystis* (LB 2385) were no different from those of a nontoxic strain (LB 2386) (Baker and Levinton unpublished data). It is unclear what proportion of *Microcystis* in the Hudson River occurred as colonies or was toxic, prior to the zebra mussel invasion.

Total phytoplankton cell densities have decreased since the invasion of the Hudson River by zebra mussels, but the proportion of diatoms has increased from 14 to 76% of the total number of cells (Marshall 1988, Smith et al. in press). Species of diatoms appear to have been affected unequally; there was a tendency for smaller genera to decline in relative abundance whereas larger diatom genera were unchanged or increased (Smith et al. in press). We found that diatoms were generally excluded from ingestion and rejected as diffuse pseudofeces. In addition, clearance rates for suspensions with diatoms, especially *Cyclotella*, were lower than those for other suspensions. Bastviken et al. (1998) also found that diatoms were cleared at net rates lower than those of other phytoplankton. These observations are consistent with the increase in relative diatom abundance in the Hudson River since the zebra mussel invasion.

Though less dramatic than the relative increase in diatoms, the proportion of green phytoplankton has also increased in the Hudson River (Smith et al. in press) from 2 to 5% since zebra mussels invaded (Marshall 1988, Smith et al. in press). Again, smaller cells tended to decline, while larger cells, such as *Scenedesmus*, increased in relative abundance (Smith et al. in press). In our study, zebra mussels generally rejected larger species of green phytoplankton from ingestion. For example, *Scenedesmus* was always rejected and clearance rates were very low when suspensions included this species. Preliminary rejection of particles such as *Scenedesmus* appears to take place on the gills, before reaching the labial palps. Using endoscopic examination and video recording, we have observed that when zebra mussels are fed *Scenedesmus* and *Microcystis* together, *Scenedesmus* moves toward the labial palps in a mucus string above the ventral food groove, while *Microcystis* moves deep within the groove and, presumably to the mouth (Baker et al. 1998).

There has been very little change in the amount of nonphytoplankton material in the Hudson River since the invasion of zebra mussels (Caraco et al. 1997). The concentration of suspended particulate matter, such as silt and detritus, is only 15% lower than the average annual load of 20 mg L⁻¹ (Cole et al. 1991, Caraco et al. 1997, Strayer et al. in press), compared to a drop in phytoplankton biomass of 90% (Caraco et al. 1997). Given this, we might expect that such particles are not removed from suspension by zebra mussels. However, we found that zebra mussels removed different particle types and particle sizes, including clay and detritus, from suspension with equal efficiency, as indicated by the lack of change in suspended particle proportion. This is not surprising, given that zebra mussels retain even 1 µm particles with greater than 90% efficiency (Sprung and Rose 1988, Roditi et al. 1996, Baker and Levinton, unpublished data). In a few cases, clay or detritus particles, rather than phytoplankton cells, were preferentially ingested by zebra mussels. We observed that pseudofeces of detritus, and especially clay particles, were easily resuspended,

even in still water. This, combined with the vigorous tidal flow of the Hudson estuary, would explain the modest drop in seston concentrations.

The changes that have taken place in the Hudson River—disappearance of cyanobacteria, increase in relative abundance of diatoms, and lack of change in turbidity—are not always typical of systems invaded by zebra mussels. For example, in Lake Erie, there has been a proportional decline in all major groups of phytoplankton (Nicholls and Hopkins 1993). In lakes and slow-moving rivers, there have been concomitant drops in turbidity, resulting in transparency increases of from 33 to 100%, presumably due to removal of clay and detritus from the water column (Holland 1993, MacIsaac and Rocha 1995). These proportional declines in particle types are consistent with observations that zebra mussels do not preferentially remove particles from suspension (Roditi et al. 1996, Bastviken et al. 1998). What then, is the underlying cause of the disproportional changes in the Hudson River? It appears that the Hudson River is sufficiently turbulent and tidally mixed to resuspend bottom material (Cole et al. 1992, Caraco et al. 1997). Therefore, particles deposited on the bottom as feces or pseudofeces are likely to be resuspended to the water column. We found that large diatoms and nonliving particles were generally rejected and that pseudofeces consisting of the these particles were very diffuse. Biodeposits of this type would be particularly susceptible to resuspension. Our study helps explain why diatoms have become the dominant phytoplankton in the Hudson River, why there has been very little change in turbidity, and why cyanobacteria, which is preferentially ingested, has disappeared since the invasion of zebra mussels.

In our study, selectivity of phytoplankton species differed, depending on the complexity of the offered suspension. For example, *Cyclotella* was preferentially ingested relative to *Thalassiosira* in combinations of three particle types, but was not preferentially accepted when paired with *Thalassiosira* only. This implies that the phytoplankton assemblage that exists before invasion is important in determining the effects that zebra mussels will have on future phytoplankton species composition. Therefore, we might expect different trajectories of phytoplankton populations in different water bodies, depending on the starting conditions. When modeling the effects of bivalve grazing on phytoplankton species composition in a given body of water, it may be necessary to include contextual selectivity measurements, as well as species-specific phytoplankton growth rates.

Our results show a general hierarchy of selectivity for nonliving particles and phytoplankton species, modulated by the specific composition of the phytoplankton species presented to the mussels. The rejection of *Typha* detritus by zebra mussels corresponds to the rejection by oysters of cord grass (*Spartina*) detritus as particles of relatively little nutritive content (Ward et al. 1998). Clay particles were ingested to a surprising degree. Previous studies (Sornin et al. 1988, Gatenby et al. 1996) show that clay may enhance bivalve growth and this may apply to zebra mussels as well.

The hierarchy of selectivity might be expected to correspond to assimilation efficiency, but few data exist to test this hypothesis. Unpublished data (H. Roditi, personal communication) show the following order of assimilation efficiency for zebra mussels: *Microcystis* > *Thalassiosira* > *Chlorella*. If we assume that selectivity of *Chlorella* is similar to our results for other green phytoplankton, then the order of assimilation efficiency corresponds generally to

our order of selectivity by *D. polymorpha*. This provides evidence that selectivity does have a reward in the degree of assimilation.

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ISOLATION AND CHARACTERIZATION OF A cDNA ENCODING AN ACTIN PROTEIN FROM THE ZEBRA MUSSEL, *DREISSENA POLYMORPHA*

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ABSTRACT A full-length complementary DNA (cDNA) encoding an actin protein was isolated from a zebra mussel testis library and subsequently sequenced. The cDNA consisted of 1,484 base pairs with an open reading frame of 1,128 base pairs. It encoded a protein of 376 amino acids with high similarity to cytoskeletal actin sequences. PCR and Northern blot indicated the presence of the mRNA for this actin in testes, ovaries, and siphons.

KEY WORDS: Actin, *Dreissena polymorpha*, mollusks, zebra mussel

INTRODUCTION

Actins are contractile and structural proteins ubiquitous in virtually all eukaryotic cells, playing a role not only in motility but also in determination of cellular shape and cytokinesis. Several isoforms have been reported in higher vertebrates, divided into muscular (class II) and cytoplasmic actins (class I) (for review see Rubenstein 1990). The actin isoforms are highly homologous, but can be distinguished by the N-terminal parts of their primary structure. The muscular pro-actin amino acid sequences specify a Met-Cys-Asp/Glu N-terminus, and the cytoplasmic actin amino acid sequences start with Met-Asp/Glu in their N-terminus. Most of the invertebrate actins thus far reported seem to be of the class II type.

The zebra mussel, *Dreissena polymorpha*, has been causing ecological and biofouling problems since its accidental introduction into the Great Lakes region of North America in the mid-1980s (Hebert et al. 1989, Ram et al. 1992). Zebra mussel populations in Lake St. Claire, believed to be the initial site of introduction, experienced a 1,000-fold increase between 1988 and 1990 (Hebert et al. 1991), and zebra mussels have since spread throughout most of the Mississippi River tributaries and the Hudson River watershed (for review see Ram & McMahon, 1996). A second exotic species of dreissenid mussels, commonly called the quagga mussel (*Dreissena bugensis*), has also appeared in the Great Lakes (Marsden et al. 1996). The rapid growth in population of the mussels is caused by their high fecundity (Borcharding 1991), and their rapid spread has been facilitated by their free-swimming larval stage, lasting up to several weeks. For these reasons there has been great interest in reproduction and early development of these mussels. Zebra mussels have been intensely studied for the last decade; however, there have been few studies on the molecular level. As a first step to molecular expression studies in the field of cell cycle and reproduction of the zebra mussel, we isolated a cDNA clone encoding actin from a testis library, which may provide a useful tool in future molecular studies.

MATERIAL AND METHODS

Animals

Sexually mature zebra mussels were collected in Lake St. Claire, Michigan. To distinguish between zebra mussels and quagga mussels, restriction fragment length polymorphism

(RFLP) in the mitochondrial cytochrome C oxidase subunit I gene (COI) was used as a diagnostic molecular marker (Baldwin et al. 1996).

RNA Isolation and Preparation of a cDNA Library

RNA was isolated from mature testes using TRIzol (Gibco BRL; Gaithersburg, MD), and poly A + RNA was purified (mRNA mini kit, Qiagen). From 5 µg of this mRNA, cDNA was synthesized using an oligonucleotide containing a poly(dT) sequence and a *XhoI* restriction site. *EcoRI* adaptors were ligated, and the cDNA was directionally cloned into *EcoRI-XhoI* sites of an Uni-ZAP XR vector (Stratagene, La Jolla, CA).

After amplification of the library, several microliters of the library were plated and eight plaques were isolated, excised *in vivo*, and partially sequenced. One clone was identified as encoding zebra mussel actin. This clone was completely sequenced in both directions with the dideoxy chain termination method (Sanger et al. 1977) using a Perkin Elmer cycle sequencing kit.

Northern Blot

RNA and mRNA were isolated from mature ovaries, mature testes, and siphon tissue as described above. mRNA was separated on a 1% MOPS/formamide gel and blotted onto a Hybond-N membrane (Amersham; Arlington Heights, IL). A 700 bp *PstI* fragment containing the N-terminal part of the actin cDNA was used as a probe in Northern blots; 25 ng of this DNA was labeled with [³²P]dATP by random priming (Random Prime, Gibco BRL). The Northern blots were prehybridized in hybridization buffer [containing 40 mM phosphate-buffered sodium (pH 7.4), 25% formamide, 6 × SSC (1 × SSC = 150 mM NaCl and 15 mM sodium citrate), 0.1% sodium dodecyl sulfate (SDS), 100 µg/mL denatured herring sperm DNA, 0.1% polyvinylpyrrolidone, 1 mM EDTA and 2 × Denhardt's solution (1 × Denhardt's solution is 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, and 0.1% Ficoll 400)], and blots were incubated with the probe in the same hybridization buffer at 42°C. After 18 h of hybridization, filters were washed twice for 30 min with 0.2 × SSC at 56°C. Autoradiography was performed (2 weeks exposure with two amplification screens at -80°C).

Reverse Transcriptase Polymerase Chain Reaction (RT PCR)

cDNA was generated from 2 µL RNA of mature ovaries, mature testes, and siphon using a superscript RT kit (Gibco BRL). Specific primers were designed for zebra mussel actin: (5' gag-gagcaccagctctattgac 3' and 5' caatgcctgggaacatgtag 3'). PCR was

The nucleotide sequence reported in this paper has been submitted to the GenBank database under the accession number: AF082863

performed on 1 μ L cDNA in 50 μ L reaction volume with 1.25 U Taq Polymerase (Gibco BRL) in 1 \times of the provided PCR buffer with a final concentration of 1.5 mM $MgCl_2$, 0.25 μ M of each primer, and 0.2 mM of dNTP mix. DNA was amplified in the Perkin Elmer Cetus thermocycler (Norwalk, CT) at 60°C with a 10-min 94°C hot start and a final extension at 72°C for 10 min. Twenty-five cycles produced a detectable amount of PCR product.

RESULTS AND DISCUSSION

Eight clones were randomly picked from the zebra mussel testis library, *in vivo* excised, and partially sequenced. One clone showed high identity with previously reported actin proteins (Fig. 1). The clone consisted of 1,484 base pairs with an open reading frame of 1,128 bp encoding a protein of 376 amino acids. The coding region

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tgcaggccggagcgaacgttttgatttttttctgacgttgagtcggacatctcttaaac      c   1
atgtgtgacgaagacgttgacgtctcttgcatgtgacaaatggatctggaatgtgcaaacgt  61
M C D E D V A A L V I D N G S G M C K A   20
gggtttgacgagacgatgtctccagagcgtgtttccctcgattgtcgccgacccaagg    181
G F A G D D A P R A V F P S I V G R P R   40
catcagggttgatggttggtatggttcagaaagacacgtatgttgagatgagggccag    241
H Q G V M V G M G Q K D S Y V G D E A Q   60
agcaagagaggtatctctcaccctcaagtaaccctatgaaacaggtatcgtaaccacgtg  301
S K R G I L T L K Y P I E H G I V T N W   80
gatgatgtggaagatctggcatcacacctctacaatgagctccgtcgctcgccacagag  361
D D M E K I W H H T F Y N E L R V A P E   100
gagcaccacgtctctatttgactgaagcccaactcaacccaaaggccaacagggaaaagatg  421
E H P V L L T E A P L N P K A N R E K M   120
accacagatcatgtttgagacctcaacaccccgacaaatgtacgtttgctatccaggcagta  481
T Q I M F E T F N T P A M Y V A I Q A V   140
ttgtcactgtatgcacccgtgtaaccactggatgtgatgagctcggtgatggtgtg    541
L S L Y A S G R T G T G I V M D S G D G   160
tctcacactgtctctatttgaaggttaacgtctctcccaacgcaattctcgctcttgac   601
S H T V P I Y E G Y A L P H A I L R L D   180
ctggtctggttagagatctacagattatctcatgaaatctcactgaacgtggtctatcca  661
L A G R D L T D Y L M K I L T E R G Y S   200
ttcacacaacacagctgaacgtgaaattgtcagagacattaaagaaaagctgtgctatggt  721
F T T T A E R E I V R D I K E K L C Y V   220
gcacttgactttgacaggaaatgcaaaatgcagccagttcaagctccttgaaaagagc    781
A L D F E Q E M Q T A A S S S S L E K S   240
tatgagctccctgacgggtcaggtcatcacaccttggaacagcagcattccgttgccagag  841
Y E L P D G Q V I T I G N E R F R C P E   260
gcaatgttccagccatctttctcgttggtatggaatctcgccgttatccatgaacacacac  901
A M F Q P S F L G M E S A G I H E T T Y   280
aacagttattgaaggtgtgatgtatgataccgtgaaagactgtgtatgcaaacactgtcctt  961
N S I M K C D V D I R K D L Y A N T V L   300
ctcgtgtggtctaccatgttccacaggtatgcogacagaatgcagaaggaaatcacagcc  1021
S G S T M F P G G I A T D R M Q K E I T A   320
cttgctcctacacagatgaagatcaagatcattgtctcccccagagagaaatcattctgtc  1081
L A P S T M K I K I I A P P E R K Y S V   340
tggaattgtgtgctccatctgctctctcgaaccttccagcagatgttgatcaagcaag    1141
W I G S I L A S L S T F Q M W I S K       360
caggagttgatgaggtggaacatccatagtcacagaaagctgtctcattatcttaaaa    1201
Q E Y D E A G P S I V H R K C F -         376
aaaactgtctaaactgcaaacccagctcagtgactggaactggatctgtttattgttgaa  1261
gtattactttattgtcagattacttaattaaagctgtgtctatcaaacagatctgtaattcg  1321
agttgtgagaaggggtgtgtcttgataggaacagttgcacgtttaattgttcaattgtgtc  1381
aattatgtgcatggtgacaaacattttcaaaataatgtggtgtgaaagaaATAAATA    1441
aaaaagctgtatttaattgttaaaaaaaataaaaaaa    1484

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Figure 1. Nucleotide and deduced amino acid sequence of a *Dreissena polymorpha* testis cDNA clone encoding actin. The nucleotides and amino acid residues are numbered from the 5' terminus of the clone and the putative initiation methionine, respectively. The regions used as primers for PCR are underlined. The polyadenylation signal is capitalized.

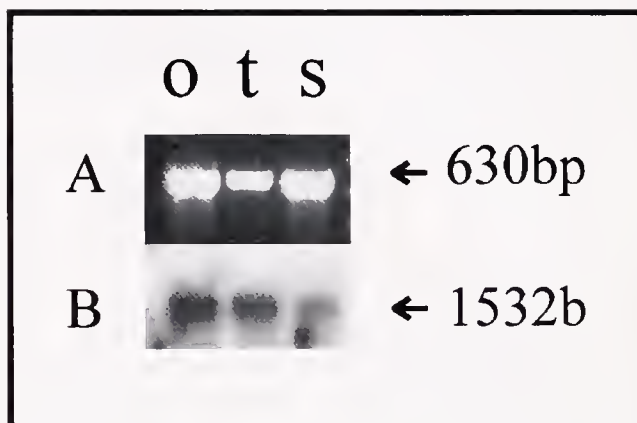


Figure 2. Presence of actin mRNA in zebra mussel tissues. (A) RT-PCR on ovary (o), testis (t), and siphon (s) mRNA using primers underlined in Figure 1. Arrows indicate DNA length markers. (B) Northern blot on ovary, testis, and siphon mRNA hybridized with a 700 bp *PstI* fragment containing the N-terminal part of the actin cDNA as a probe.

was flanked by 61 nontranslated bases at the 5' end and 287 bases at the 3' end. A putative polyadenylation site (-AATAAA-) was found 21 bases upstream from the poly(A) tail. RT-PCR and Northern blot analysis of mRNA from various tissues demonstrated the presence of actin mRNA in testes, ovaries, and siphons (Fig. 2).

The predicted amino acid sequence of the coding region showed 97.1% identity and 99.5% similarity with the cytoskeletal actin isoform of *Aplysia* (DesGroseillers et al. 1994), its closest match. In the latter mollusk a muscular actin isoform has been reported as well (DesGroseillers et al. 1990), showing 95.2% identity with its nonmuscular counterpart. However, in the sea scallop *Placopecten magellanicus* (Patwary et al. 1996), an actin isoform isolated from muscular tissue shows higher similarity to cytoskeletal actin than to the muscular isoform.

As in most of the other invertebrates, the first N-terminal amino acids of the zebra mussel actin sequence are Met-Cys-Asp-(MCD-), typical for the muscular actins in vertebrates (Rubinstein 1990). Nevertheless, when considering the entire amino acid sequence, the zebra mussel actin sequence is more similar to class II (cytoskeletal) vertebrate actins than to the vertebrate muscular actins. Although it is not clear whether bivalves have both isoforms, we presume that the zebra mussel clone reported here has cytoskeletal functions. Furthermore, this zebra mussel actin clone may provide a useful tool in molecular studies of reproduction, early development, and other aspects of zebra mussel biology.

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PHYSICAL FACTORS THAT LIMIT THE DISTRIBUTION AND ABUNDANCE OF *DREISSENA POLYMORPHA* (PALL.)

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ABSTRACT *Dreissena polymorpha*, the zebra mussel, originated in the Caspian and Black Sea basin, and has invaded waterbodies of the Former Soviet Union (FSU), eastern, and western Europe during the last 200 years. Since 1771 more than 2,000 papers on the distribution, taxonomy, biology, ecology, and control of species and subspecies of *Dreissena* were published in Russia and FSU, however, this work has not been generally available. We review work conducted in the FSU over the last 100 years on the tolerance of *D. polymorpha* to different abiotic conditions in waterbodies of various types in order to provide non-Russian-speaking scientists access to this rich body of literature. *D. polymorpha* is one of the few bivalve molluscs well adapted to live from entirely fresh water to brackish waters. Different subspecies appear to have different salinity tolerances, ranging from completely fresh water to 18.5‰. Zebra mussels require at least 25% oxygen saturation, although they can survive several days in anaerobic conditions. The quagga mussel, *D. bugensis*, however, is much more tolerant of low oxygen conditions. The upper temperature limit for zebra mussels is around 32–34°C. Zebra mussels are most abundant on hard surfaces, particularly rocky surfaces, and on macrophytes. Water motion, aerial exposure, freezing, and the other physical factors including temperature, salinity, and oxygen affect the distribution of zebra mussels among and within waterbodies. Many of these factors are expected to covary, or differ predictably among waterbodies, driving distribution and abundance patterns as well as patterns of invasibility that we see both within and among waterbodies.

KEY WORDS: Zebra mussels, salinity, oxygen tolerance, temperature, substrate

INTRODUCTION

Invading exotic species are presently considered to be one of the greatest threats to native ecosystems throughout the world (Vitousek 1994). One of the best known recent aquatic invaders to North America is the zebra mussel, *Dreissena polymorpha* and to a lesser extent, the quagga mussel, *Dreissena bugensis*. Because of the large environmental and economic impacts of the *D. polymorpha* invasion in the Great Lakes region and major rivers of eastern USA and Canada, scientists and natural resource managers across Canada and the USA, as well as other countries that have not yet been invaded, are attempting to prevent and plan for likely further invasion of this bivalve. Although *Dreissena* species have recently invaded North America (Hebert et al. 1989), *D. polymorpha* has been an aggressive invader in fresh waters across countries of the former Soviet Union (FSU), and eastern and western Europe for 200 years, following the activities of humans, especially the building of canals, shipping, and commercial fishing (Starobogatov and Andreeva 1994, Karatayev et al. 1997). Given this long history, there has been a great deal of research in eastern and western Europe on species and subspecies of *Dreissena*. Due to language and political barriers, however, work performed and published in the FSU has only recently become available to western and English-speaking scientists (listed in Schloesser et al. 1994, reviewed in Karatayev et al. 1997).

In a recent review, Karatayev et al. (1997) summarized work conducted in eastern Europe and the FSU on the effects of *D. polymorpha* invasion on aquatic communities, and where possible, contrasted these results with current and ongoing research in North America. In this review, our goal is similar. We review work that has been conducted in the FSU addressing physical factors that influence the presence and abundance of dreissenid mussels and

how those factors could affect distribution in different types of waterbodies. We present this review to familiarize scientists with this body of work and, where possible, contrast this work with work conducted in North America. This should not, however, be considered a comprehensive review of North American literature. We hope that familiarity with past work from the FSU will help science progress faster; using an existing body of research and literature allows more sophisticated questions to be addressed than would be possible if we knew nothing about the biology of *Dreissena*. In addition, comparing and contrasting results of research from the FSU with work from North America is valuable. If findings are similar, it strengthens our ability to make generalizations, and if findings are different, we can begin to determine what environmental or other factors are responsible for such differences.

In this review we examine several specific physical factors, including salinity, oxygen, and temperature, that are known to affect the presence and abundance of dreissenids. We then address how these factors affect the differences in abundance of dreissenids among different types of waterbodies. Although we present these factors individually, they are not necessarily independent. Many factors will covary, and there are likely to be interactions among factors that have a much greater impact on dreissenids than would be seen by examining factors individually. In addition, some factors may be more common in certain environments, contributing to between-habitat differences observed. However, to date there is no dataset available in a form where multifactor analysis can be used to address the impacts of all of these specific factors (Ramcharan et al. 1992a). Hopefully, as attention is drawn to the need for such data and as work progresses, specific data will become available allowing this type of analysis.

Because of differences between English and Russian language

alphabets, and no reliable convention for the translation of letter combinations or words between these two languages, there may be differences among publications in the spelling of author names and English titles of works. In this work, whenever possible, we use standard terminology and spelling, and use American library standards for the titles of journals (Alkire 1969a,b).

SALINITY

Dreissena bugensis, which has invaded some, but not all of the Laurentian Great Lakes but has not been important invader in FSU or the rest of Europe (Karatajev et al. 1997), naturally occurs in waters with salinities $<5‰$ (Starobogatov and Andreeva 1994). In contrast, *D. polymorpha* is found in a wide range of salinities (Kinzelbach 1992, Shkorbatov et al. 1994, Starobogatov and Andreeva 1994, Lyakhnovich et al. 1994). However, it appears that there are several subspecies of *D. polymorpha*, each with a more narrow but different tolerance to salinity (Logvinenko and Starobogatov 1968, Galperina and Lvova-Kachanova 1972, Kinzelbach 1992, Lyakhnovich et al. 1994, Rosenberg and Ludyanskiy 1994, Shkorbatov et al. 1994, Starobogatov and Andreeva 1994) (Table 1). Thus, *D. polymorpha polymorpha* and *D. polymorpha andrusovi* populate the Caspian Sea (Logvinenko and Starobogatov 1968, Galperina and Lvova-Kachanova 1972, Lyakhnovich et al. 1994, Shkorbatov et al. 1994, Starobogatov and Andreeva 1994). *D. polymorpha obtusicarinata* (Andr.) and *D. polymorpha aralensis* (Andr.) are widespread in the Aral Sea (Khusainova 1954, Khusainova 1958, Yablonskaya 1960a, Yablonskaya 1960b, Zenkevich 1963, Mordukhai-Boltovskoi 1972, Yablonskaya et al. 1973, Starobogatov and Andreeva 1994). Natural distributions along salinity gradients, distribution changes due to human-caused

changes in salinity, and direct experimental testing are all consistent with the salinity tolerance of individual subspecies and support the notion that different subspecies have different salinity tolerances. Therefore, although as a species *D. polymorpha* has a wide salinity tolerance, from fresh to $14‰$, each subspecies is more restricted in its distribution.

Dreissena polymorpha polymorpha

D. polymorpha polymorpha has been the most successful invader in the FSU, Europe, and North America, and has the lowest salinity tolerance of any of the subspecies of *Dreissena* (Lvova 1977, Kinzelbach 1992, Starobogatov and Andreeva 1994). In the Caspian Sea (Karpevich 1964, Shkorbatov et al. 1994) and the Taganrog Gulf of the Azov Sea (Karpevich 1955b, Kruglova 1957, Shkorbatov et al. 1994) *D. polymorpha polymorpha* populates the least saline areas, from fresh water up to $5‰$ (Table 1).

Several examples illustrate the link between salinity tolerance and the distribution of *D. polymorpha polymorpha*. The highest densities and standing biomass (up to $1\text{--}2\text{ kg m}^{-2}$) of *D. polymorpha polymorpha* in the Asov Sea are found below $2.5‰$ (Karpevich 1955b, Stark 1955, Yablonskaya 1955, Nekrasova 1971, Lyakhnovich et al. 1994). Prior to 1950 the Don River flowed directly into the Azov Sea resulting in large areas of low salinity, and zebra mussels were wide spread (Mordukhai-Boltovskoi 1937, Nekrasova 1971, Nekrasova 1973). After construction of the Tsimslyanskoe Reservoir (1952) on the Don River, and the subsequent reduction of the water flow to the Azov Sea, salinity increased, and in low-water years *D. polymorpha polymorpha* is only found near the Don River Delta. In high-water years the salinity is lower, and zebra mussels occupy the whole eastern part of the Taganrog Gulf of the Azov Sea (Nekrasova 1971, Nekrasova 1973, Lyakhnovich et al. 1994).

Even within *D. polymorpha polymorpha*, different populations can have different salinity tolerances. For example, Markovskiy (1954) concluded that there were different populations of *D. polymorpha polymorpha* with different salinity tolerances within the Dnieper Bug Liman (a salty coastal lake). One population lives in salinities of $0.2\text{--}3.0‰$ and the other in areas with salinity $0.5\text{--}5.0‰$. For other populations, the maximum density (up to $100,000\text{ m}^{-2}$) and biomass (up to 3 kg m^{-2}) of *D. polymorpha polymorpha* and *D. bugensis* are in areas with low salinity (average 0.8 , maximum $2.0\text{--}3.5‰$) (Grigoryev 1965).

Similar patterns have been found in the Netherlands. Lake IJsselmeer was formed in 1932 by closing its connection to the Baltic Sea (Smit et al. 1993). Chloride concentrations gradually dropped from $6‰$ in 1932 to $0.6‰$ in 1935, and to $0.4‰$ in 1936. *D. polymorpha polymorpha* was first recorded in Lake IJsselmeer in 1936, and by 1937 were found in the northern (lower salinity) part of the lake, and by 1938 were distributed throughout the whole lake. The same pattern was observed in Lakes Volkerakmeer and Zoommeer (Smit et al. 1993). These lakes were dammed from the Eastern Scheldt Estuary in April 1987, and chloride concentrations dropped from $10‰$ to $0.8‰$ by September 1987. The first adult mussels were found in Lake Volkerakmeer in October 1987. In January 1988, *D. polymorpha polymorpha* had spread throughout Lake Volkerakmeer. Lake Zoommeer has higher chloride concentrations ($0.7‰$) than Lake Volkerakmeer ($0.3\text{--}0.4‰$), and was colonized 2 years later. The higher chloride concentration is suggested to be the reason for slower invasion in this lake (Smit et al. 1993).

TABLE 1.

Upper salinity limit for different subspecies of *Dreissena polymorpha* found in various waterbodies.

Waterbody	Upper Salinity Limit (‰)	References
<i>D. polymorpha polymorpha</i>		
Dnieper-Bug Liman	5	Markovskiy 1954
Netherlands estuaries	4	Wolff 1969
Vistula lagoon	4.8	Klimovich 1958
Rhine estuaries	5	Remane and Schlieper 1958
North-Baltic Sea Canal	6.2	Reshott 1961
Northern part of the Caspian Sea	5	Karpevich 1964
Azov Sea	5	Karpevich 1955b
	5	Kruglova 1957
	5	Shkorbatov et al. 1994
<i>D. polymorpha andrusovi</i>		
Caspian Sea	11	Karpevich 1952a
	12	Ivanova 1973
	12–14	Shkorbatov et al. 1994
	13	Logvinenko 1965
	13	Lvova 1988
	13	Lvova and Makarova 1990
<i>D. polymorpha aralensis</i>		
Aral Sea	17.6	Khusainova 1958
<i>D. polymorpha obtusicarinata</i>		
Aral Sea	18.4	Lyakhnovich et al. 1994

An experimental study of the salinity tolerance of *D. polymorpha polymorpha* in the upper reaches of the Volga River (Kuibyshev and Kostroma reservoirs) showed that the salinity threshold at which 50% of animals are physiologically stressed and stop filtering, is about 1‰ (Antonov and Shkorbatov 1983). In contrast, the 50% stress threshold for zebra mussels from the Volga River Delta is 4.7–4.8‰ (Antonov and Shkorbatov 1983). Salt tolerance of populations decreases with increasing distance from the Caspian Sea, and has been suggested to be based on genetic differences (Antonov and Shkorbatov 1983, Antonov and Shkorbatov 1984, Shkorbatov 1986, Shkorbatov and Antonov 1986, Shkorbatov et al. 1994). However, definitive long-term experiments to determine if these populations level differences are due to acclimation or acclimatization still need to be conducted. In laboratory experiments to test the effects of rapid changes in salinity on *D. polymorpha polymorpha* it was found that rate of salinity change affects salinity tolerance; lower salinities are lethal when change is rapid than when there is a gradual increase in salinity (Karpevich 1947a, Karpevich 1947b, Karpevich 1952a, Karpevich 1955a, Shkorbatov et al. 1994).

Thus, in Europe, *D. polymorpha polymorpha* can live in salinities up to 6‰ (Table 1). North American studies have found similar salinity limitations (Kilgour et al. 1994, Walton 1996). Given the salinity tolerances of this subspecies, it should be able to spread widely into estuaries and brackish waters in North America as has been predicted by Strayer and Smith (1993). However, a salinity limit of 10–14‰, suggested by these authors, is high. Only *D. polymorpha andrusovi*, *D. polymorpha obtusicarinata* and *D. polymorpha aralensis* have been found at such high salinities in other parts of the world.

Dreissena polymorpha andrusovi

D. polymorpha polymorpha and *D. polymorpha andrusovi* are both found in the Northern Caspian Sea (Logvinenko and Starobogatov 1968, Galperina and Lvova-Kachanova 1972, Lyakhnovich et al. 1994, Shkorbatov et al. 1994, Starobogatov and Andreeva 1994). For both subspecies, benthic distribution and biomass are inversely correlated with salinity (Vinogradov 1959, Osadchikh 1963, Lyakhnovich et al. 1994). The salinity of the Caspian Sea increased more than 10‰ from 1937 to 1940 and, as a consequence, the biomass and distribution of both *D. polymorpha polymorpha* and *D. polymorpha andrusovi* were dramatically reduced (Karpevich 1947a, Karpevich 1952a, Karpevich 1952b, Vinogradov 1959, Osadchikh 1963, Lyakhnovich et al. 1994). Subsequently, when the salinity was reduced, abundance increased and reached previous levels by the end of the 1960s. The salinity of the Northern Caspian increased again, and by the end of the 1970s was >10‰, causing another drastic decrease in the biomass and cover (Lyakhnovich et al. 1994).

Although both subspecies occupy similar habitats, they differ in some of their habitat requirements. Especially high densities of *D. polymorpha andrusovi* are found on silty-shell sediments in the eastern and southern areas of the Northern Caspian Sea (Starobogatov and Andreeva 1994), and *D. polymorpha andrusovi* has a higher salinity tolerance than *D. polymorpha polymorpha*, up to 11–13‰ (Table 1). Different field studies have found different salinity optima for *D. polymorpha andrusovi*, ranging from 5 to 10‰ (Karpevich 1947b), 3 to 7‰ (Vinogradov 1955), and 3 to 8‰ (Lyakhnovich et al. 1994).

In laboratory experiments, optimal salinities for *D. polymorpha*

andrusovi was the same for mussels from either low (4‰) or high (10‰) salinity habitats and ranged from 2 to 12‰ (Shkorbatov et al. 1994). *D. polymorpha andrusovi* can survive for a short time at salinities from 0 to 17‰, and even at extremes of 19 to 20‰. However, *D. polymorpha andrusovi* cannot form stable populations in waters <2‰ or >12–14‰ (Shkorbatov et al. 1994).

Interestingly, the distribution of *D. polymorpha andrusovi* in other regions of the Caspian Sea has sharply decreased due to the invasion of another mussel, *Mytilaster lineatus* (Gm.). This mussel was accidentally introduced into the Caspian Sea from the Black Sea in the 1920s with motorboats transported by train during the Russian Civil War (Logvinenko 1965). *M. lineatus* has a greater tolerance of hypoxia and appears to have out competed *D. polymorpha andrusovi* (Starobogatov and Andreeva 1994).

Dreissena polymorpha obtusicarinata and *Dreissena polymorpha aralensis*

D. polymorpha obtusicarinata and *D. polymorpha aralensis* have higher salinity tolerance than other subspecies of *D. polymorpha*, and at the end of the 1950s were the dominant benthic animals in the Aral Sea (Khusainova 1954, Khusainova 1958, Yablonskaya 1960a, Yablonskaya 1960b, Zenkevich 1963, Mordukhai-Boltovskoi 1972, Yablonskaya et al. 1973, Starobogatov and Andreeva 1994). *D. polymorpha obtusicarinata* was found in salinities up to 18.4‰ (Lyakhnovich et al. 1994). According to Zenkevich (1963), the average historic salinity of the Aral Sea during natural hydrological conditions was 10.2‰. Intensive use of water from rivers (Amu-Darya and Syr-Darya) flowing into the Aral Sea for irrigation caused a dramatic increase (up to 28‰) in the salinity (Kotlyakov 1991). Subsequent to increased water salinity since the 1960s, the abundance and density of all subspecies of *Dreissena* has decreased (Mordukhai-Boltovskoi 1972, Yablonskaya et al. 1973). An especially dramatic reduction in *D. polymorpha obtusicarinata* was observed in the mid-1970s, and now this subspecies is believed to be completely extinct (Andreeva and Andreev 1990b, Bekmurzaev 1991, Starobogatov and Andreeva 1994).

D. polymorpha aralensis was found near estuaries of the Amu-Darya and Syr-Darya Rivers and in waterbodies of the Aral Sea drainage basin (Starobogatov and Andreeva 1994). Experimental studies and field observations demonstrated that adult *D. polymorpha aralensis* can survive in salinities from 0.6 to 17.6‰, and its larvae can survive from 2.0 to 17.6‰ (Khusainova 1958). By 1980, after an increase in salinity, *D. polymorpha aralensis* disappeared from the Aral Sea (Andreeva and Andreev 1990b, Starobogatov and Andreeva 1994); however, it may still be found in other waterbodies of the Aral Sea drainage basin (Starobogatov and Andreeva 1994).

Unfortunately, the effects of physical factors other than salinity on the different subspecies of *D. polymorpha* have not been extensively studied, and the majority of research has been conducted on *D. polymorpha* from freshwater habitats, and therefore, *D. polymorpha polymorpha*. Unless otherwise indicated, the remainder of the literature we review is research on *D. polymorpha polymorpha*.

TEMPERATURE

The lower temperature limit for *D. polymorpha* is 0°C; zebra mussels cannot survive freezing (Luferov 1965). Mikheev (1967a) studied the filtering activity of zebra mussels from the Pyalovskoe Reservoir (Russia) at low water temperatures. He found that latero-

frontal cilia of gill filaments are completely motionless at 0.5°C, and the lateral cilia barely move at that temperature. The activity of the cilia increases with rising temperatures. At 2°C some of the latero-frontal cilia become active, but not all; active rows of latero-frontal cilia are adjacent to nonmoving cilia on the same gill. At 3°C all of the cilia become active (Mikheev 1967a). Reeders and Bij de Vaate (1990) also reported an increase in filtering rate at 3°C.

Although filtering initiates at a low temperature, growth and development in *D. polymorpha* require much higher temperatures. Many studies have found that 10°C is the minimal temperature for growth and development in *D. polymorpha* (Lvova-Kachanova 1972, Alimov 1974, Lvova 1977, Karatayev 1983, Lyakhnovich and Karatayev 1988, Mackie 1991, Karatayev and Lvova 1993, Jantz and Neumann 1992, Lyakhnovich et al. 1994). Morton (1969b, 1969c) found that *D. polymorpha* grows only when water temperature is >11°C.

Field studies have shown that spawning in *D. polymorpha* is initiated at 15–18°C (Kachanova 1961, Kachanova 1962, Kirpichenko 1964, Puchkova and Polivannaya 1967, Hillbricht-Ilkowska and Stanczykowska 1969, Kirpichenko 1971a, Kirpichenko 1971b, Kirpichenko 1971c, Lvova-Kachanova 1972, Galperina 1976, Skalskaya 1976, Suter-Weider and Zimmermann 1976, Lvova 1977, Stanczykowska 1977, Lvova 1980, Karatayev 1981, Lewandowski 1982a, Karatayev 1983, Stanczykowska et al. 1983a, Karatayev 1988, Karatayev and Lvova 1993, Lvova et al. 1994). Similar temperature thresholds have been observed in North American studies (Fraleigh et al. 1993, Mackie 1991, Mackie 1993, Leach, 1993, Garton and Haag 1993).

In contrast to spawning, European and North American studies have found that veligers can be found in the plankton when temperatures exceed 12°C (Shevtsova 1968a, Wiktor 1969, Sloka 1969, Kornobis 1977, Sprung 1987, Sprung 1989, Borchering 1991, Sprung 1991, Borchering 1992, Neumann et al. 1993, Sprung 1993, Ram et al. 1993), and some authors report finding larvae at 8–9°C (Yaroshenko and Naberezhny 1971, Kachalova and Sloka 1964). However, spawning of *D. polymorpha* at temperatures lower than 10°C is doubtful, particularly as zebra mussels do not appear to grow or develop below 10°C. Karatayev (1983) suggested that veligers observed at low temperatures might have been carried downstream by currents from warmer parts of reservoirs or connected waterways.

Natural field experiments on the effects of temperature on the growth and reproduction of zebra mussels have been provided by research conducted in the cooling reservoirs of thermal and nuclear power plants. Heating can increase the growth rate and productivity of *D. polymorpha* (Stanczykowska 1976, Elagina et al. 1978, Karatayev 1983, Karatayev 1984, Karatayev 1990, Lvova et al. 1994). The density and biomass of *D. polymorpha* are usually higher in areas of cooling reservoirs where water temperatures are 2–6°C above natural temperatures (up to 30°C) than they are in areas where temperatures are not elevated (Pidgaiko 1974, Mordukhai-Boltovskoi 1975, Soszka and Soszka 1976, Korgina 1978, Karatayev 1983, Grigelis and Raciunas 1984, Karatayev 1988). For example, the production/biomass coefficient for zebra mussels in areas heated 1.5–5°C above normal by the Lukomlskaya Thermal Power Station is 50% higher, and the average seasonal growth is 57–100% higher than for zebra mussels grown in unheated waters (Karatayev 1983, Karatayev 1984, Karatayev 1990, Lvova et al. 1994). In addition, *D. polymorpha* starts spawning earlier in the season in waterbodies warmed by cooling waters (Karatayev

1981, Lewandowski and Ejsmont-Karabin 1983). Thus, in the heated zone of the Konin Lakes, *D. polymorpha* reproduction begins in April, but not for another 2 months in the unheated zone (Lewandowski and Ejsmont-Karabin 1983). In heated areas of cooling reservoirs larvae are also found in the plankton longer than in unheated areas (Kornobis 1977, Lewandowski and Ejsmont-Karabin 1983, Stanczykowska et al. 1988), suggesting that spawning takes place over a longer period.

Numerous studies have been conducted in both the field (in cooling reservoirs of thermal and nuclear power stations) and laboratory to determine the upper temperature limit for adult *D. polymorpha* (Shkorbatov 1981, Shkorbatov and Antonov 1980, Karatayev 1983, Protasov et al. 1983a, Protasov et al. 1983b, Antonov and Shkorbatov 1984, Shkorbatov and Antonov 1986, Shkorbatov 1986, Afanasiev and Protasov 1987, Antonov and Shkorbatov 1990, Afanasiev and Shatkhina 1993, Shkorbatov et al. 1994, Antonov 1997). Laboratory studies conducted by Shkorbatov et al. are the most detailed. They carried out long-term studies of zebra mussel tolerance to high temperatures from different regions of the FSU (Shkorbatov and Antonov 1980, Shkorbatov 1981, Antonov and Shkorbatov 1984, Shkorbatov 1986, Shkorbatov and Antonov 1986, Antonov and Shkorbatov 1990, Shkorbatov et al. 1994, Antonov 1997). In the laboratory they found that the temperature threshold where 50% of the population is stressed (do not filter, but effects are reversible, P_t 50%) for different populations of *D. polymorpha* from Volga River varied from 24.8 to 28.1°C, and the 100% thermal stress threshold (P_t 100%) is between 28–31°C. Lethal temperatures for the populations studied, where 50% of mussels die (L_t 50) and where 100% of mussels die (L_t 100) are 30.7–33.0 and 33.5–36.0°C, respectively (Shkorbatov et al. 1994). These authors found that the upper temperature limit for zebra mussels depends on the climatic zone from which they were collected. The highest thermal tolerance has been observed for *D. polymorpha* from the Volga Delta (46°N), and the lowest for zebra mussels from the Rybinskoe Reservoir (59°N) (Antonov and Shkorbatov 1984, Shkorbatov and Antonov 1986, Shkorbatov 1986, Shkorbatov et al. 1994). Within a waterbody, higher thermal tolerance ($\pm 1^\circ\text{C}$) was found for *D. polymorpha* from heated areas as compared with unheated areas of the Kostromskaya Thermal Power Station (Russia) cooling reservoir (Shkorbatov and Antonov 1986, Shkorbatov et al. 1994).

Upper temperature limits for zebra mussels observed in the field rather than the lab might be a better predictor of geographic limits to *D. polymorpha* spread. Afanasiev (1986) found that zebra mussels can be inhibited by temperatures higher than 27°C, and even short exposure to 29°C can eliminate more than 70% of a *D. polymorpha* population (Afanasiev et al. 1988). However, many authors have shown that *D. polymorpha* can live at $\geq 30^\circ\text{C}$ (Karatayev 1983, Protasov et al. 1983b, Vladimirov 1983, Karatayev 1984, Afanasiev and Protasov 1987, Lyakhnovich and Karatayev 1988, Karatayev 1992, Afanasiev and Shatkhina 1993, Karatayev and Lvova 1993, Protasov and Sinitsina 1993, Sinitsina and Protasov 1993, Lyakhnovich et al. 1994, Aldridge et al. 1995), and can survive in cooling reservoirs at temperatures up to 32–34°C (Table 2).

The most detailed field studies of thermal tolerance of zebra mussels in FSU were conducted in Lukomskoe Lake, a cooling reservoir for the Lukomskaya Thermal Power Station. Experiments were conducted using caged zebra mussels placed in three different areas: the unheated area of the reservoir (zone III), the area with moderate heating (1.5–4.9°C above normal, zone II), and

TABLE 2.
Upper temperature limit for *Dreissena polymorpha* in various waterbodies.

Waterbody	Temperature (°C)	References
Adult zebra mussels		
Lukomskoe Lake	32	Karatayev 1983
Zaporozhskoe Reservoir	31.5	Lyakhnovich et al. 1994
Canal of the Pridneprovskaya Power Station	32	Lyakhnovich et al. 1994
Kuchurganskiy Liman	32	Vladimirov 1983
Cooling reservoir of the South-Ukrainian Nuclear Station	33	Sinitsina and Protasov 1993
Cooling reservoir of the Chernobyl Nuclear Station	34	Protasov et al. 1983b
Veligers		
Konin Lakes	29	Lewandowski and Ejsmont-Karabin 1983
Canal Dnieper-Krivoi-Rog	30	Shevtsova 1968a

the area with the highest degree of heating (4.8–10.8°C above normal, zone I) (Karatayev 1983, Karatayev 1984, Karatayev 1992) (Table 3). Mortality was greatest when temperatures exceeded 32°C (Tables 2, 3). In general, zebra mussels survived temperatures up to 31–34°C (Table 2). In North America, zebra mussels from rivers have been shown to have thermal limits of around 30–32.5°C (Aldridge et al. 1995). Zebra mussel larvae appear to be less tolerant of high temperature than adults (Shevtsova 1968a, Lewandowski and Ejsmont-Karabin 1981) (Table 3).

D. bugensis appears to be less tolerant of high temperatures than *D. polymorpha* (Dyga and Zolotareva 1976, Antonov and Shkorbatov 1990, Domm et al. 1993). Dyga and Zolotareva (1976) found that in the heated zone of the Zaporozhskoe Reservoir, quagga mussels can survive up to 30.5°C, lower than the tolerance of *D. polymorpha* (Table 2).

OXYGEN

The tolerance of dreissenids to low oxygen conditions has been the focus of many field and laboratory studies (Ovchinnikov 1954,

Feigina 1959, Mikheev 1967a, Mikheev 1967b, Spiridonov 1971, Shkorbatov 1981, Antonov and Shkorbatov 1984, Shkorbatov and Antonov 1986, Shkorbatov et al. 1994). Initially, Zhadin (1946) suggested that *D. polymorpha* do not occur in habitats where the oxygenation falls below 91% of full saturation. Later Ovchinnikov (1954) found, in the Rybinskoe Reservoir, that *D. polymorpha* live in areas with oxygen content higher than 70–80%, and Feigina (1959) found that *D. polymorpha* can survive in waters with oxygen concentration not less than 50% of full saturation. More recent work has shown a critical threshold of 25% oxygenation for *D. polymorpha* (Mikheev 1961, Spiridonov 1972, Shkorbatov et al. 1994). However, zebra mussels can survive for several days in anoxic conditions, depending on the temperature. Mikheev (1964) found that 100% of *D. polymorpha* in anaerobic conditions died on the sixth day of exposure at 17–18°C, on the fourth day at 20–21°C, on the third day at 23–24°C, and that small mussels were more sensitive to the lack of oxygen. Karpevich (1952a) found that *D. polymorpha* in anoxic conditions at 17–18°C can survive 4–5 days. Spiridonov (1972) reported 100% mortality of zebra mussels in anoxic conditions at 20°C after 6–7 days. Similar results have

TABLE 3.

Mortality of *Dreissena polymorpha* in experimental cages in the most heated (I), medium heated (II), and unheated (III) zones of Lukomskoe Lake (from Karatayev 1983).

Zone	Size range (mm)	Initial sample size May 9	Monthly mortality					Total mortality	
			June 9	July 28	August 6	September 8	October 28	Number of individuals	%
I	8–10	23	0	0	19	2	1	22	96
	14–16	27	0	1	20	3	0	24	89
	20–22	28	1	1	25	1	0	28	100
	25 & >	24	1	2	20	1	0	24	100
	T °C	16.6	29.2	26.8	32.4	25.8	16.9		
II	8–10	26	0	0	1	0	0	1	4
	14–16	27	1	0	0	0	0	1	4
	20–22	27	1	0	0	0	1	2	7
	25 & >	20	1	1	0	0	0	2	10
	T °C	9.8	22.8	23.5	24.9	18.3	12.9		
III	8–10	25	1	0	0	0	0	1	4
	14–16	27	1	0	1	0	0	2	7
	20–22	27	0	0	0	0	0	0	0
	25 & >	28	0	0	0	0	0	0	0
	T °C	8.0	21.0	22.0	21.6	16.0	8.0		

been obtained for *D. polymorpha* from the Kuibyshevskoe Reservoir (Shkorbatov et al. 1994).

D. bugensis appears to be more tolerant of low oxygen conditions than *D. polymorpha*. Although Shkorbatov et al. (1994) found that all *D. polymorpha* died under anoxic conditions at the fourth day of exposure, all *D. bugensis* survived through the fourth day. Birger et al. (1975) also showed that *D. polymorpha* requires higher oxygen concentrations than *D. bugensis*.

Observations in the field of the distribution of *D. polymorpha* support laboratory findings. In the Zaporozhskoe Reservoir, zebra mussels are never found in deep waters, where the oxygen content near the bottom decreases to 3–5% of full saturation during the summer season (Lyakhnovich et al. 1994). Because of high oxygen requirements, *D. polymorpha* cannot survive in or above silts, which actively absorb oxygen (Lvova 1978, Lyakhnovich et al. 1994). In the Kuibyshevskoe Reservoir (Russia), *D. polymorpha* grows on 5.5 m submerged dead trees that were stranded when the reservoir was created by flooding a forest. It does not grow on the bottom 1 m of trees over the silty sediment (Lyakhov and Mikheev 1964). High densities of zebra mussels overgrow trees to the very bottom at another site in the same reservoir with a constant water current and oxygenation (Lyakhov and Mikheev 1964).

Construction of a cascade of reservoirs on the Dnieper River (Ukraine) changed the hydrological regime in the Zaporozhskoe and Dnieprovskoe reservoirs, increasing siltation and decreasing oxygen levels. *D. bugensis* replaced *D. polymorpha* as the dominant species in these reservoirs because it has a higher tolerance for silt and low oxygen, and now 80–90% of the mussels in these two reservoirs are *D. bugensis* (Zhuravel 1967, Birger et al. 1968, Lubyantsev and Zolotareva, 1976). The density and biomass of quagga mussels in the Zaporozhskoe Reservoir are as high as 100,000–130,000 m⁻² (10–12 kg m⁻²) (Dyga and Zolotareva 1976). Similar patterns were observed in other reservoir cascades in the Ukraine, where *D. bugensis* displaces *D. polymorpha* as the dominant species (Zhuravel 1965, Zhuravel 1967, Birger et al. 1968, Zagubizhenko and Lubyantsev 1971, Birger et al. 1975, Pligin 1979).

SUBSTRATE

One of the main factors that affects the distribution and abundance of *D. polymorpha* is suitable substrate for attachment (Zhadin 1946, Mordukhai-Boltovskoi 1960, Karatayev 1983, Lyakhnovich and Karatayev 1988, Lyakhnovich et al. 1994, Karatayev and Burlakova 1995a). In most lakes, rock and sometimes coarse sand can be the most suitable substrate for zebra mussel attachment (Zhadin 1948, Lubyantsev 1957, Zhuravel 1967, Khusainova 1958, Zhuravel 1959, Lyakhov 1961, Gasunas 1965, Grigoryev 1968, Gontya 1971, Kirpichenko and Lyakhov 1976, Draulans and Wouters 1988, Karatayev and Burlakova 1995a, Nalepa et al. 1995, Burlakova 1998) (Table 4). However, in shallow parts of large lakes and reservoirs, even on suitable substrates, particularly sands, zebra mussels can be limited by water motion if the water motion is great enough to dislodge mussels or moves the sediment and mussels, stranding them above water or burying them (Lvova 1977, Karatayev 1983, Lyakhnovich et al. 1994, Karatayev and Burlakova 1995a, MacIsaac 1996, Burlakova 1998). Shelly sediments (Berg 1938, Mordukhai-Boltovskoi 1960, Stankovic 1960, Kuznetsov 1970, Nekrasova 1971, Lvova 1977, Karatayev and Tishchikov 1979, Karatayev 1983, Lyakhnovich and Karatayev 1988, Bij de Vaate 1991, Lyakhnovich et al. 1994), silty sand

(Yablonskaya 1955, Khusainova 1958, Mordukhai-Boltovskoi 1960, Gavrilov et al. 1976, Kovaleva 1969, Karatayev 1983, Lyakhnovich and Karatayev 1988, Lyakhnovich et al. 1994), and submerged portions of macrophytes (Feigina 1950, Khusainova 1958, Grigoryev 1965, Butenko 1967, Shapkarov and Angelovski 1978, Lewandowski 1982b, Karatayev 1983, Lyakhnovich and Karatayev 1988, Lyakhnovich et al. 1994, Karatayev and Burlakova 1995a, Burlakova 1998) are also suitable substrates for *D. polymorpha*.

Zebra mussels can be extremely abundant on submerged macrophytes (Khusainova 1958, Yablonskaya 1960b, Gontya 1971, Dyga and Lubyantsev 1975, Lvova, 1977, Lvova 1978, Lewandowski 1982b, Lewandowski 1983, Karatayev 1983, Lyakhnovich and Karatayev 1988, Lyakhnovich et al. 1994, Karatayev and Burlakova 1995a). The longevity of macrophyte stems or fronds is usually shorter than the life span of zebra mussels. When macrophyte stems die back, *D. polymorpha* falls to the bottom and usually die, especially when the lake bottom under the macrophytes is covered by mud with little oxygenation (Gaidash and Lubyantsev 1975, Lewandowski 1982b, Karatayev 1983, Lyakhnovich et al. 1994, Burlakova 1998). Therefore, zebra mussels on macrophytes are usually much younger than on other substrates (Yablonskaya 1960b, Gaidash and Lubyantsev 1975, Lewandowski 1982b, Karatayev 1983, Lewandowski 1983, Lyakhnovich et al. 1994, Burlakova 1998). Submerged macrophytes with perennial stems or fronds (*Chara* sp., *Fontinalis* sp., *Elodea canadensis*, *Ceratophyllum* sp.) are colonized by *D. polymorpha* up to 3 years old, whereas plants with stems that die back annually (*Myriophyllum spicatum*, *Potamogeton* sp.) have only yearling individuals (Stanczykowska and Lewandowski 1980, Lewandowski 1982b).

The poorest substrate for zebra mussels is silt (Zhadin 1946, Kachanova 1963, Biryukov et al. 1964, Nekrasova 1971, Lvova 1977, Karatayev 1983, Draulans and Wouters 1988, Lyakhnovich and Karatayev 1988, Lyakhnovich et al. 1994, Karatayev and Burlakova 1995a, Burlakova 1998). However, *D. polymorpha* can live on silty sediments if plant fragments, wood, shells, or stones are available for attachment (Zhadin 1946, Zhadin 1948, Mordukhai-Boltovskoi 1960, Nekrasova 1971, Karatayev 1983). Zebra mussels can use the hard fragments for initial attachment and subsequently can attach to each other forming druses (Lubyantsev 1960, Mordukhai-Boltovskoi 1960, Karatayev 1983, Lyakhnovich and Karatayev 1988, Lyakhnovich et al. 1994).

Similar results have been found in North America. Although *D. polymorpha* has been reported to colonize areas dominated by silt in the Laurentian Great Lakes (Hunter and Bailey 1992, Dermott and Munawar 1993), Hunter and Bailey (1992) found that *D. polymorpha* colonized soft substrates in Lake St. Clair by lateral extension of druses which originated from attachment to small pieces of hard substrate, usually live unionid mussels, unionid shells, or clusters of zebra mussels.

High densities of zebra mussels may also be found in newly constructed reservoirs where the bottom is covered with only a thin layer of silt (Kovaleva 1967, Kovaleva 1969, Spiridonov 1971, Mikheev and Novik 1971, Kirpichenko and Lyakhov 1976, Kovaleva 1973). As reservoirs age and silt builds up, zebra mussel densities decline (Lyakhov and Mikheev 1964, Kirpichenko 1968, Mikheev and Novik 1971, Lvova 1977). After the construction of the reservoir for the Kaunas Hydroelectric Power Plant (Lithuania) in 1961, rocky areas were abundant and zebra mussels were found at high densities. By 1972, 80% of the bottom was covered by silt, resulting in a dramatic decrease in zebra mussel density (Bubinas

TABLE 4.

Density and biomass of *Dreissena polymorpha* on various substrates in different types of waterbodies.

Substrate	Waterbody	Density mean \pm SE (m ⁻²)	Biomass mean \pm SE (g \cdot m ⁻²)	References
Stones	Sara Lake	1,580	642	Lyakhnovich et al. 1994
	Naroch Lake 1997	2396 \pm 469	548 \pm 107	Burlakova 1998
	Myastro Lake 1995	1644 \pm 698	1024 \pm 491	Burlakova 1998
	Reservoir Drozdy 1995	5540 \pm 1729	2154 \pm 568	Burlakova 1998
Sand	Volgogradskoe Reservoir	118	5	Kovaleva 1969
	Lukomskoe Lake	1,374	527	Karatayev 1983
	Naroch Lake 1997	211 \pm 42	57 \pm 11	Burlakova 1998
	Myastro Lake 1995	1911 \pm 683	969 \pm 448	Burlakova 1998
	Batorino Lake 1995	474 \pm 382	85 \pm 58	Burlakova 1998
	Reservoir Drozdy 1995	977 \pm 326	409 \pm 151	Burlakova 1998
Silty sand	Volgogradskoe Reservoir	870	32	Kovaleva 1969
	Lukomskoe Lake	3,930	967	Karatayev 1983
Shells	Lukomskoe Lake	2,607	854	Karatayev 1983
	Sara Lake	1,585	825	Lyakhnovich et al. 1994
	Naroch Lake 1997	1081 \pm 218	251 \pm 62	Burlakova 1998
Semisubmerged macrophytes	Lukomskoe Lake	2,577	684	Karatayev 1983
	Batorino Lake 1995	2,276 \pm 1,023	1,163 \pm 314	Burlakova 1998
Submerged macrophytes	Lukomskoe Lake	3,545	369	Karatayev 1983
	Sara Lake	1,248	309	Lyakhnovich et al. 1994
	Naroch Lake 1997	3,171 \pm 417	275 \pm 31	Burlakova 1998
	Myastro Lake 1995	1,523 \pm 294	193 \pm 70	Burlakova 1998
Clay	Volgogradskoe Reservoir	72	19	Kovaleva 1969
Silty soil	Volgogradskoe Reservoir	657	25	Kovaleva 1969
Pure silt	Lukomskoe Lake	2	0.1	Karatayev 1983
	Naroch Lake 1997	64 \pm 64	15 \pm 15	Burlakova 1998
	Myastro Lake 1995	0	0	Burlakova 1998
	Batorino Lake 1995	65 \pm 61	20 \pm 20	Burlakova 1998
	Drozdy Reservoir 1995	0	0	Burlakova 1998
	Kuibyshevskoe Reservoir	3,150	1,860	Lyakhov and Mikheev 1964
	Sylvenskiy bay of Kamskoe Reservoir	60,803 ^a	21,605	Gubanova 1969
Flooded forests	Volgogradskoe Reservoir	22,000	4,000	Spiridonov 1967
	Volgogradskoe Reservoir	—	1,480	Spiridonov 1971
	Volgogradskoe Reservoir	4,660 ^a	—	Konstantinov & Spiridonov 1977

^a Not including yearling mussels.

1980). A pronounced decrease of *D. polymorpha* density with increasing silt was also found in lakes of Volga River Delta (Pokrovskaya 1966). Zebra mussels were the dominant species in these lakes in the 1920s, but completely disappeared by the end of the 1950s due to a dramatic increase in bottom siltation. Tseyeb et al. (1966) found in Dnieper River reservoirs that *D. bugensis* is more adapted to live on silt sediments than *D. polymorpha*. Low densities of zebra mussels can also be found on clay (Kovaleva 1969, Dyga and Lubyantsev 1975, Lyakhnovich et al. 1994).

WATERBODIES OF DIFFERENT TYPES

Lakes

Zebra mussels can be found in a wide range of types of waterbodies, however, most work has focused on factors affecting their presence and abundance in lakes. These have included the importance of factors such as nutrients, pH, and calcium (Stanczykowska 1977, Ramcharan et al. 1992a, Ramcharan et al. 1992b, Lyakhnovich et al. 1994, and references there in), biotic factors

(Molloy et al. 1997, and references there in), as well as lake morphometry (Strayer 1991). Using data from European lakes, Ramcharan et al. (1992a) found apparent thresholds of calcium carbonate and pH that determine the presence or absence of zebra mussels, and that high nutrient levels are associated with lower population densities. Ramcharan et al. (1992b) also found that lake size and flushing rate had an impact on population fluctuations of zebra mussels in European lakes. Based on data from Europe, Strayer (1991) suggested that small warm lakes are more suitable for *D. polymorpha* than deep and cold lakes.

Research in the FSU has found that trophic type affects the probability of finding zebra mussels in a lake (Ovchinnikov 1933, Deksbakh, 1935, Karatayev 1989, Lyakhnovich et al. 1994, Karatayev and Burlakova 1995a, Karatayev and Burlakova 1995b). Ovchinnikov (1933) and Deksbakh (1935) suggested that eutrophic lakes are the best habitat for *D. polymorpha*. Using data from 553 Belarussian lakes, Karatayev and associates (Karatayev 1989, Karatayev and Burlakova 1995a, Karatayev and Burlakova 1995b, A. Karatayev, L. Burlakova, L. Johnson and D. Padilla,

unpublished data) have shown that zebra mussels are found most often in mesotrophic lakes, are found less often in oligotrophic and meso-oligotrophic lakes, least often in eutrophic lakes, and do not inhabit dystrophic lakes. However, as Karatayev et al. (1997) point out, the presence of zebra mussels in a lake will decrease chlorophyll levels, lowering the trophic status of a lake subsequent to invasion. Better information about the trophic status of lakes when they are initially invaded will help answer this question.

Studies on the effects of trophic status on zebra mussels have shown that the maximum density of *D. polymorpha* shifts to shallower depths with increasing lake eutrophication (Karatayev and Burlakova 1995a, Burlakova 1998). Moreover, the maximum depth of zebra mussels within a lake increases with decreased trophic status. One possible mechanism for this pattern is that the decrease in water clarity, with increased eutrophication, reduces the depth of submerged macrophytes, which are an important substrate for zebra mussels. In addition, increased eutrophication leads to increased siltation in lakes, reducing the area of suitable substrates. This results in shifting the *D. polymorpha* distribution to shallower parts of lakes (Karatayev and Burlakova 1995a, Burlakova 1998). These hypotheses can be tested as we gather more comparable information for more lakes with different trophic status.

Reservoirs

In reservoirs formed by the damming of rivers, *D. polymorpha* colonizes all suitable substrates, often at high densities (Zhuravel 1952, Lyakhov 1961, Kovaleva 1967, Lyakhov 1967, Kovaleva 1973, Mordukhai-Boltovskoi et al. 1974, Lyakhov and Mordukhai-Boltovskoi 1976, Lvova 1977, Lvova 1980, Lyakhovich et al. 1994). For example, *D. polymorpha* colonized the Dnieprovskoe Reservoir, formed by damming the Dnieper River, during the first few years after its construction in 1932 (Zhuravel 1952). During the Second World War the dam was removed when the Soviet army retreated in 1941, and the density of *D. polymorpha* in the former reservoir portion of the Dnieper River dramatically decreased. After reconstruction of the reservoir, *D. polymorpha* again attained high densities (Zhuravel 1952).

Especially high densities of *D. polymorpha* form in reservoirs created by flooding forested areas, because zebra mussels colonize flooded stumps, trunks, and branches of trees and brushwood (Zhuravel 1952, Luferov 1963, Lyakhov and Mikheev 1963, Lyakhov and Mikheev 1964, Gromov 1965, Mordukhai-Boltovskoi and Dzuban 1966, Luferov, 1966, Lyakhov 1967, Spiridonov 1967, Gubanova 1968, Gubanova 1969, Spiridonov 1970, Spiridonov 1971, Andronova et al. 1976, Kirpichenko and Lyakhov 1976, Konstantinov and Spiridonov 1977, Lvova 1977, Spiridonov 1978, Lyakhovich et al. 1994) (Talbe 1). In the flooded areas of the Kuibyshevskoe Reservoir (Russia) which did not have trees, *D. polymorpha* was found in 46% of samples at an average density of 518 m⁻², whereas in the areas that were flooded forests, zebra mussels were found in 80% of samples at an average density of 3,150 m⁻² (Lyakhov and Mikheev 1964). In the Volgogradskoe Reservoir (Russia) the highest density (22,000 m⁻²) and biomass (4 kg m⁻²) of *D. polymorpha* are also found in flooded forest areas (Spiridonov 1967, Spiridonov 1970), and in the Sylvenskiy Bay of the Kamskoe Reservoir, the density of *D. polymorpha* in flooded forest areas was as high as 371,703 m⁻² with a biomass of 11.4 kg m⁻² (Gubanova 1968).

In reservoirs that are drawn down during the winter, *D. poly-*

morpha disappears from the shallow areas (Kachanova 1963, Mikheev 1964, Gromov 1965, Kirpichenko 1968, Mikheev and Novik 1971, Konstantinov and Spiridonov 1977, Lvova 1977, Lvova 1980, Pligin 1978, Lyakhovich et al. 1994). Zebra mussels attached to large invertebrates, such as unionid mussels, that migrate to deeper waters survive (Mikheev and Novik 1971, Lyakhovich et al. 1994). Thus, the shallow areas of reservoirs subject to draw downs or fluctuating water levels are usually inhabited only by yearling mussels. In the Uchinskoe Reservoir (Russia) this area extends to a depth of 1.5 m (Lvova 1977, Lvova 1978), and, in the Kremenchugskoe Reservoir (Ukraine), down to 2 m, and covers 18% of the reservoir area (Lyakhovich et al. 1994). However, Mikheev and Novik (1971) found that zebra mussels can survive in the shallow drained areas under ice for long periods at temperatures near 0°C, but not lower. They found that zebra mussels died only in the parts of reservoirs that drained in the autumn, before ice cover.

Extremely high densities of *D. polymorpha* have also been found on various artificial substrates in reservoirs (Protasov et al. 1983a, Afanasiev and Protasov 1987, Toderash and Vladimirov 1990, Lyakhovich et al. 1994). For example, on the concrete walls of the cooling reservoir of the Chernobyl Nuclear Power Station (Ukraine), the density of *D. polymorpha* was 248,000 m⁻², and biomass was 12 kg m⁻² (Protasov et al. 1983a). This extremely high density of zebra mussels formed due to both the presence of suitable artificial substrate and constant water currents.

Rivers

In rivers, zebra mussels are most affected by unidirectional water flow, disturbance due to water flow, suspended sediment, and minimal suitable substrates for attachment. Frequently, large molluscs (bivalves and gastropods) will be the most abundant hard substratum for zebra mussel attachment in rivers, and are some of the most often used substrates for attachment in any type of waterbody (Sebestyen 1937, Zhadin 1948, Zhuravel 1957, Zhuravel 1959, Lubyantsev 1956, Zhadin and Gerd 1961, Wiktor 1963, Kachalova and Sloka 1964, Kuchina 1964, Butenko 1967, Wolff 1969, Mikheev and Novik 1971, Lewandowski 1976, Elagina et al. 1978, Karatayev 1983, Karatayev and Tischikov 1983, Tischikov 1984, Arter 1989, Lyakhovich et al. 1984, Lyakhovich et al. 1994, Karatayev and Burlakova 1995a, Ricciardi et al. 1996, Karatayev et al. 1997). Often, unionid mussels are the only suitable substrata for zebra mussel attachment in rivers (Zhadin 1948, Lubyantsev 1956, Lubyantsev 1960, Kachalova and Sloka 1964, Kuchina 1964, Tischikov 1984, Lyakhovich et al. 1984, Lyakhovich et al. 1994). For example, in the Severnaya Dvina River the density of zebra mussels is low (<10 m⁻²), and zebra mussels are found only on unionid mussels and occasional stones (Kuchina 1964). Similarly, zebra mussels are found most frequently on unionid mussels in the Daugava River (Kachalova and Sloka 1964) and Berezina River (Lyakhovich et al. 1984).

Other factors preventing zebra mussels from attaining high densities in rivers are the movement of bottom sediments and high concentrations of suspended matter, especially during periodic flooding (Zhadin 1946, Zhadin 1959, Lubyantsev 1956, Lubyantsev 1960, Kuchina 1964, Kondratiev and Spiridonov 1970, Stanczykowska 1977, Nedostup 1988, Lyakhovich et al. 1994). Khushainova (1954, 1958), and Mordukhai-Boltovskoi (1960) found that zebra mussels are rare in rivers with constant high concentrations of suspended matter, as it inhibits filtering activity.

Unidirectional water flow makes it difficult for local populations of zebra mussels in rivers to increase in density, as larvae are swept downstream. However, high densities of *D. polymorpha* can form in rivers flowing from lakes or reservoirs populated by zebra mussels (Lubyanov 1957, Kirpichenko 1963, Lyakhnovich et al. 1984, Clevlen and Frenzel 1993, Karatayev and Burlakova 1995a) which provide a supply of larvae and colonizing juveniles.

High densities of zebra mussels can also form in the lower courses of rivers and deltas because of very slow flow, and reduced movement of bottom sediments (Kuchina 1964, Grigoriev 1965, Kharchenko and Shevtsova 1983, Lyakhnovich et al. 1994). In the lower Danube River the average density of *D. polymorpha* is 1,000 m⁻² and the biomass is 185 g m⁻² (Kharchenko and Shevtsova 1983). Extremely high densities of zebra mussels are also found in the South Bug Liman (Ukraine) (Grigoriev 1965), where in the shallow areas the density of zebra mussels on semisubmerged macrophytes (*Pragmites communis*) is 169,000 m⁻², and biomass is 3–5 kg m⁻², but at other sites without macrophytes, the density is 1,730 m⁻², and biomass is 1,240 g m⁻².

Canals

Canals are distinct from lakes and reservoirs because there is a constant, unidirectional water current, and differ from rivers because bottom sediments are much more stable and the concentration of suspended matter is much lower (Karatayev 1983, Burlakova 1998). Extremely high densities of zebra mussels form in canals (Kachanova 1962, Kachanova 1963, Puchkova and Polivannaya 1967, Shevtsova 1968b, Lvova-Kachanova 1971, Kaftanikova 1975, Kornobis 1977, Lvova 1977, Stanczykowska 1977, Karatayev 1983, Sokolova et al. 1981, Shevtsova and Kharchenko 1981, Kharchenko and Shevtsova 1983, Afanasiev 1987, Lyashenko and Kharchenko 1988, Lyakhnovich et al. 1994). The average biomass of zebra mussels in the Uchinskoe Reservoir (Russia) was 117–1,044 g m⁻², in the canal outflowing from this reservoir, the average biomass was 3–5 kg m⁻² (Lvova 1977). In canals connecting the Konin Lakes (Poland), which are heated, the average density of zebra mussels was 40,000 m⁻², in the lakes the densities varied from 100 to 1,100 m⁻² (Kornobis 1977). In Lukomskoe

Lake (Belarus) the average density of zebra mussels in 1978 was 758 m⁻², with a biomass of 124 g m⁻², whereas in the canal outflowing from this lake the average density of zebra mussels was 58 times, and biomass was 55 times higher (Karatayev 1983). The maximum density of *D. polymorpha* in the North-Crimean Canal was 19,899 m⁻², and biomass was 4.7 kg m⁻² (Shevtsova and Kharchenko 1981). Extremely high densities (30,000 m⁻²), and biomass (50 kg m⁻²) of zebra mussels were found on the walls of the Dnieper-Donbass Canal as well (Kharchenko and Shevtsova 1983).

DISCUSSION AND GENERAL FINDINGS

Physical factors affect the distribution and abundance of zebra mussels among and within waterbodies, often in predictable patterns (Table 5). Important driving factors affecting the distribution of zebra mussels include suitable substrate, oxygen content, salinity, and temperature extremes. Factors affecting population density and growth are temperature and suitable substrate for attachment. Lethal temperatures (below 0°C and above 30°C) control zebra mussel distribution, as do critical temperatures for growth and spawning.

Abiotic factors such as desiccation, salinity, and oxygen can set limits to the distribution of *D. polymorpha* and its many subspecies. Some of these factors vary in a predictable fashion. Desiccation can happen at the surface of waterbodies whose water level fluctuates, either naturally as with rivers and lakes, or due to human control as with reservoirs and canals. Salinity in a liman, estuary, or small sea is greatly affected by the influx of fresh water either through rainfall (climatic conditions) or the control of rivers and canals. Increases in salinity decrease *D. polymorpha* abundance and survivorship. Oxygen content of waters can be affected by many factors. Oxygen content will decrease with increased temperature and metabolic activity of living organisms, including microbes. High rates of primary productivity or decomposition and low rates of water flow can create bottom waters with very low oxygen content as is seen in eutrophic lakes and reservoirs. Oxygen content can also be affected by sediment type, as some sedi-

TABLE 5.
Physical factors limiting *Dreissena polymorpha* populations in various waterbodies.

Factor	Type of Waterbody			
	River	Lake	Reservoir	Canal
Sediment motion	High	Low (could limit in shallow areas only)	Low (could limit in shallow areas only)	Low
Suspended inorganic matter	High	Low (could limit in shallow areas only)	Low (could limit in shallow areas only)	Low
Low O ₂ stress	Low	Medium (could limit in profundal zone and in highly eutrophic lakes)	Medium (could limit in profundal zone)	Low
Suitable substrate limitation	High—depends on availability of molluscs and macrophytes	High—varies with depth More suitable substrates usually shallow	High	High—except where artificial substrates are used
Water level variation	Medium	Low	High in shallow areas	Low
Low temperature	Low	High in profundal area only	High in profundal area and shallow areas with freezing	Low
Transport of larvae downstream	High	None	None	High

ments such as silts can actively adsorb oxygen, reducing its content in surrounding waters.

As a result of the abiotic factors discussed, the minimum depth where zebra mussels are found is from 0.1 to 0.5 m, depending on local water level fluctuations and the probability of freezing (Stanczykowska 1976, Karatayev 1983, Karatayev 1988, Lyakhovich and Karatayev 1988, Lyakhovich et al. 1994, Burlakova 1988). The abundance of suitable substrate for attachment also affects depth distributions of zebra mussels. Many studies have found that in lakes and reservoirs zebra mussels have a maximum density at depths from 1 to 5 m (Kachanova 1963, Lvova-Kachanova 1971, Lvova-Kachanova and Izvekova 1973, Wishniewski 1974, Stanczykowska 1975, Stanczykowska et al. 1975, Lvova 1976, Stanczykowska 1977, Lvova 1978, Karatayev 1983, Stanczykowska et al. 1983b, Karatayev 1988, Lyakhovich and Karatayev 1988, Lyakhovich et al. 1994, Karatayev and Burlakova 1995a). However, when suitable substrate and water oxygen conditions occur deeper, as in Bodensee (Germany), the maximum density of *D. polymorpha* was recorded between 5–15 m depth (Grim 1971), and at 18 m in Lake Garda (Italy) where the density was $24,000 \text{ m}^{-2}$ (Franchini 1978). In Lake Erie, maximum density ($3,443 \text{ m}^{-2}$) and total wet biomass (340.9 g m^{-2}) of zebra mussels were found at 10–20 depth (Dermott and Munawar 1993).

In the profundal depths of lakes, zebra mussels are usually limited by lack of suitable substrate for attachment and low oxygen (Lubyantsev 1965, Lvova 1979, Karatayev 1983, Lyakhovich and Karatayev 1988, Lyakhovich et al. 1994, Karatayev and Burlakova 1995a, Burlakova 1988). In places with suitable substrate and good oxygen conditions, zebra mussels spread down to greater depths (Shapkarov 1969, Walz 1973, Franchini 1978, Starobogatov and Andreeva 1994). In Garda Lake, zebra mussels spread down to 50 m (Franchini 1978) and in Bodensee to 55 m (Walz 1973). *D. polymorpha andrusovi* inhabits the Caspian Sea down to 200–300 m (Mordukhai-Boltovskoi 1960, Starobogatov and Andreeva 1994). However, in the hypolimnion of lakes, zebra mussels never attain high densities, even on suitable substrate, because of low temperatures for growth and lower oxygen conditions (Grim 1971, Walz 1973, Walz 1978a, Walz 1978b).

In the Laurentian Great Lakes, dreissenids occur at extreme depths. In Lake Erie, *D. polymorpha* and *D. bugensis* are relatively abundant in the profundal zone (Dermott and Munawar 1993). In general, the proportions of quagga mussels, which are more tolerant of low oxygen conditions, increase with depth whereas zebra mussels decrease (Zhuravel 1957, Zhuravel 1959, Zhuravel 1967,

Mackie 1991, Mills et al. 1993, Dermott and Munawar 1993). In Lake Ontario, both quagga and zebra mussels coexist at depths of 8–110 m in varying proportions, with only the quagga mussel found at 130 m (May and Marsden 1992, Mills et al. 1993). Because there are no similar lakes to the Laurentian Great Lakes in the FSU, we have no similar data to compare.

In shallow parts of large lakes and reservoirs zebra mussels can be limited by movement of consolidated sand or stone sediments and high concentrations of suspended matter (Lvova 1977, Lyakhovich et al. 1994). *D. polymorpha* seems unable to survive in waterbodies with constant concentrations of suspended matter greater than $10\text{--}40 \text{ g m}^{-3}$ (Mikheev 1967b). However, zebra mussels can live where the concentration of suspended matter is periodically greatly increased, such as in the Taganrog Gulf of the Azov Sea, where the content of suspended matters periodically increases (during storms) up to 500 g m^{-3} (Lyakhovich et al. 1994).

Physical factors are important in determining both the distribution and abundance of zebra mussels. Many of these factors, acting alone or in combination, are responsible for the patterns that we see in nature, both within and among waterbodies. Further research is required to determine how these factors may covary or act in concert to affect the biology, distribution, and abundance of zebra mussels. Our goal has been to facilitate access to the large body of scientific research that has been conducted over several decades in the FSU on zebra mussels and physical factors that affect their distribution and abundance. Hopefully, access to this research will allow us to move forward to find generalizations, and where appropriate, determine differences in the causal factors affecting the distribution and abundance of zebra mussels.

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GENETIC RELATIONSHIPS OF SEVERAL AMBLEMINI SPECIES (BIVALVIA: UNIONIDAE) IN ARKANSAS

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ABSTRACT Allozyme analysis of 16 loci was utilized to determine the genetic relationships for four species of mussels in the Tribe Amblemeni (*Amblesma plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa pustulosa* (L. Lea), and *Q. quadrula* (Rafinesque)). Conspecific genetic distance was quite low between populations for each species, with ranges of 0.012–0.113. The congeneric distance for *Q. p. pustulosa* and *Q. quadrula* was determined to be 0.333. Distances between genera ranged from 0.396 to 0.787, similar ranges to those of other unionid species studied. Genetic distance of *Amblesma* and *Plectomerus* was greater than that expected based on previous studies, with *Plectomerus* more closely related to *Quadrula* than to *Amblesma*.

KEY WORDS: Unionidae, genetic analysis, allozyme

INTRODUCTION

The freshwater bivalves of the family Unionidae are a rich taxonomic group with longstanding difficulties in classification. A historical reliance on conchological features, which exhibit a high degree of phenotypic plasticity, has obscured the existence of convergent and parallel evolution (Kat 1983a, Davis 1984). Attempts to clarify higher taxonomic relationships have resulted in the usage of various soft anatomical and reproductive features for taxonomic analysis (Heard and Guckert 1971, Davis and Fuller 1981, Kat 1983b). More recently, immunologic and allozyme analyses have been utilized (Davis and Fuller 1981, Davis et al. 1981, Kat 1983a, Kat 1983b, Kat and Davis 1984, Stevens and Alderman 1992). Davis et al. (1981) found close genetic relatedness by way of allozyme analysis among species possessing radically different shell morphologies and geographic distributions.

The tribe Amblemeni, endemic to North America, is characterized by eight genera and 27 species (Davis and Fuller 1981, Davis 1984, Williams et al. 1993). The tribe Amblemeni is characterized by relatively old genera having lineages persisting more than 10 million years (Davis et al. 1981). Genetic distances between genera should therefore be intermediate between more recent groups such as the Pleurobemini and ancient groups, such as the Margaritiferinae and Anodontini (Davis 1984). The genera within this tribe originated as early as 10 million years ago (Davis et al. 1981).

This study utilized cellulose acetate electrophoresis and histochemical staining of allozymes to determine the genetic distance for three genera and four species of the tribe Amblemeni. *Amblesma plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa pustulosa* (L. Lea), and *Q. quadrula* (Rafinesque) are dominant community members within the Cache and White rivers, which belong to the Mississippi River drainage system (Christian 1995). Each species studied is widely distributed within the Mississippi River drainage (Williams et al. 1993). No Arkansas mussel populations have been previously studied by allozyme analysis.

Davis (1984) identified a high genetic distance between genera of the Amblemeni. However, on the basis of immunologic and morphologic features, Davis and Fuller (1981) placed the genera *Amblesma*, *Megaloniaia*, and *Plectomerus* as congeneric. Our primary objective in this study was to test the genetic relationships of

these species through biochemical allozyme analysis. Since Christian (1995) and Posey (1997) reported that occasional individuals collected in Arkansas streams possess intermediate anatomic characteristics between *Q. p. pustulosa* and *Q. quadrula*, a secondary objective was to determine the genetic distance between these two species which may be hybridizing.

MATERIALS AND METHODS

Genetic distance was determined within populations for the lower regions of the Cache and White rivers in Arkansas. Three sequential downstream mussel beds were chosen for each river: miles 37, 36, and 35 (sites A, B, and C, respectively) for the Cache River, and miles 63.5, 57.2, and 48.5 for the White River (sites D, E, and F, respectively). Four species of bivalves were studied, with 12–36 individuals of two species collected from each site. Hookah rig diving was used to obtain the mussels in the summer of 1994. Mussels were brought back to the laboratory on ice and processed immediately or frozen at -70°C . Voucher specimens have been deposited in the Unionacea collection of the Arkansas State University Museum of Zoology (ASUMZ).

Adductor muscles were homogenized in equal volumes (w/v) of Tris-HCl buffer (pH 7.0). Electrophoresis of homogenate was performed on cellulose acetate plates at 200 volts for 15 min in TG buffer (0.025 M Tris; 0.192 M Glycine) at room temperature (2 mA/plate). Nine enzyme systems representing 16 loci were selected for analysis based upon their expression in adductor muscle. The enzymatic loci were as follows: fumarase (FUM-1, FUM-2; E.C. No. 4.2.1.2); glutamate-oxaloacetate transferase (GOT-1, GOT-2; E.C. No. 2.6.1.1); isocitrate dehydrogenase (IDH-1, IDH-2; E.C. No. 1.1.1.42); lactate dehydrogenase (LDH-1, LDH-2; E.C. No. 1.1.1.27); malate dehydrogenase (MDH-1, MDH-2; E.C. No. 1.1.1.37); malic enzyme (ME-1, ME-2; E.C. No. 1.1.1.40); mannose phosphate isomerase (MPI-1, MPI-2; E.C. No. 5.3.1.8); phosphoglucose isomerase (PGI-1; E.C. No. 5.3.1.9); and phosphoglucosylmutase (PGM-1, PGM-2; E.C. No. 2.7.5.1) (IUBNC 1984). The distance of migration for each specific enzyme was visualized by histochemical staining (Hebert and Beaton 1989).

Individual genotypes were used as original data with allele frequencies, locus heterozygosity, genetic identity, and Nei's unbiased genetic distance determined using the program BIOSYS-1 (Swofford and Selander 1989).

TABLE 1.

Allele frequencies, mean direct count heterozygosities (H), and polymorphism (P) of each of the four species at each site^a

Locus	Population											
	AP-A	AP-B	PD-A	PD-B	PD-C	QP-D	QP-E	QP-F	QQ-D	QQ-E	QQ-F	QQ-C
FUM-1												
(N)	35	23	31	12	36	12	20	15	32	24	36	36
A	0.500	0.826	0.048	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.500	0.174	0.935	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FUM-2												
(N)	35	1	31	18	36	12	20	15	32	24	36	36
A	0.771	1.000	0.968	1.000	1.000	0.500	0.975	0.867	0.047	0.000	0.000	0.000
B	0.143	0.000	0.032	0.000	0.000	0.500	0.025	0.133	0.953	1.000	1.000	1.000
C	0.086	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GOT-1												
(N)	35	24	31	18	36	12	20	15	32	24	36	36
A	0.357	0.000	0.113	0.167	0.000	0.000	0.050	0.000	0.063	0.104	0.000	0.000
B	0.614	1.000	0.790	0.694	1.000	1.000	0.950	0.933	0.938	0.458	1.000	1.000
C	0.029	0.000	0.097	0.139	0.000	0.000	0.000	0.067	0.000	0.438	0.000	0.000
GOT-2												
(N)	35	24	31	18	36	12	20	15	32	24	36	36
A	0.029	0.000	0.839	0.972	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.100	0.000	0.161	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.800	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDH-1												
(N)	35	23	31	18	36	12	20	14	32	24	36	36
A	1.000	1.000	1.000	1.000	1.000	0.667	0.975	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.333	0.025	0.000	0.000	0.000	0.000	0.000
IDH-2												
(N)	35	23	31	18	36	12	16	15	32	24	36	36
A	0.943	1.000	0.968	1.000	1.000	1.000	1.000	1.000	0.000	0.021	0.000	0.000
B	0.057	0.000	0.032	0.000	0.000	0.000	0.000	0.000	1.000	0.979	1.000	1.000
LDH-1												
(N)	0	0	0	0	0	11	20	15	32	24	36	36
A	0.000	0.000	0.000	0.000	0.000	0.636	0.525	0.600	0.906	0.875	0.764	0.792
B	0.000	0.000	0.000	0.000	0.000	0.364	0.475	0.400	0.031	0.042	0.167	0.208
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.083	0.069	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-2												
(N)	0	0	1	1	1	10	17	13	32	24	36	36
A	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-1												
(N)	35	24	30	18	36	12	20	15	32	24	36	36
A	0.129	0.167	0.267	0.361	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.529	0.500	0.467	0.444	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.329	0.292	0.267	0.194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.014	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MDH-2												
(N)	35	24	31	18	36	12	20	15	32	24	36	36
A	0.000	0.042	0.806	0.500	1.000	1.000	0.950	0.933	1.000	1.000	1.000	1.000
B	0.914	0.958	0.194	0.500	0.000	0.000	0.050	0.067	0.000	0.000	0.000	0.000
C	0.086	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ME-1												
(N)	34	23	27	18	36	12	20	15	32	24	36	35
A	0.206	0.130	0.222	0.167	0.000	0.000	0.050	0.033	0.344	0.104	0.139	0.000
B	0.206	0.217	0.704	0.722	1.000	1.000	0.925	0.967	0.656	0.625	0.861	0.986
C	0.485	0.522	0.074	0.111	0.000	0.000	0.025	0.000	0.000	0.271	0.000	0.014
D	0.103	0.130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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TABLE 1.
continued

Locus	Population											
	AP-A	AP-B	PD-A	PD-B	PD-C	QP-D	QP-E	QP-F	QQ-D	QQ-E	QQ-F	QQ-C
ME-2												
(N)	35	24	31	18	36	12	20	15	32	24	36	36
A	0.886	0.875	0.032	0.000	0.000	0.500	0.100	0.000	1.000	1.000	1.000	1.000
B	0.114	0.125	0.903	0.833	1.000	0.500	0.875	0.933	0.000	0.000	0.000	0.000
C	0.000	0.000	0.065	0.167	0.000	0.000	0.025	0.067	0.000	0.000	0.000	0.000
MPI-1												
(N)	35	24	31	18	36	12	20	15	32	24	36	36
A	0.329	0.000	0.113	0.667	1.000	1.000	1.000	0.967	0.000	0.646	0.028	0.000
B	0.671	1.000	0.758	0.333	0.000	0.000	0.000	0.033	1.000	0.354	0.917	1.000
C	0.000	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
PGI-1												
(N)	35	24	31	18	36	12	20	15	32	24	36	36
A	1.000	1.000	0.935	1.000	1.000	0.167	0.425	0.000	0.750	0.792	0.806	0.375
B	0.000	0.000	0.065	0.000	0.000	0.833	0.575	1.000	0.250	0.208	0.194	0.028
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.597
PGM-1												
(N)	35	22	31	18	36	12	20	15	32	24	36	36
A	0.843	0.977	0.403	0.278	0.431	0.000	0.125	0.167	0.000	0.167	0.000	0.000
B	0.157	0.023	0.468	0.722	0.569	1.000	0.875	0.833	0.328	0.417	0.194	0.542
C	0.000	0.000	0.129	0.000	0.000	0.000	0.000	0.000	0.609	0.333	0.792	0.458
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.083	0.014	0.000
PGM-2												
(N)	35	19	1	1	1	11	18	15	29	21	34	20
A	0.629	1.000	0.000	0.000	0.000	0.500	0.444	0.300	0.293	0.571	0.574	0.575
B	0.257	0.000	0.000	0.000	0.000	0.500	0.528	0.067	0.483	0.214	0.132	0.425
C	0.114	0.000	0.000	0.000	0.000	0.000	0.028	0.433	0.224	0.214	0.250	0.000
D	0.000	0.000	1.000	1.000	1.000	0.000	0.000	0.167	0.000	0.000	0.044	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.000
H (dc)	0.167	0.064	0.042	0.056	0.049	0.188	0.128	0.117	0.062	0.069	0.060	0.041
(SD)	0.081	0.044	0.024	0.023	0.049	0.092	0.057	0.057	0.033	0.028	0.031	0.027
P	0.857	0.286	0.769	0.538	0.077	0.375	0.500	0.438	0.375	0.438	0.375	0.250

^a *A. p. plicata* (AP), *P. dombeyanus* (PD), *Q. p. pustulosa* (QP), and *Q. quadrula* (QQ) in three collecting sites in the Cache (A, B, C) and White rivers (D, E, F), Arkansas.

RESULTS

Allele Frequencies

Allele frequencies were determined for each of the four species at each site. Twelve polymorphic loci were identified for *A. p. plicata* out of the 14 loci studied from sites A and B in the Cache River (Table 1). Ten polymorphic loci were identified for *P. dombeyanus* out of 13 loci in sites A, B, and C of the Cache River. *Plectomerus* individuals from site C exhibited a very high degree of monomorphism, with only PGM-1 polymorphic. Six of the 16 loci studied for *Q. p. pustulosa* were monomorphic for each of the three sites (D, E, and F) in the White River. *Quadrula quadrula* had fewer polymorphic loci (7) than did *Q. p. pustulosa* (10). Three populations (sites D, E, and F) of *Q. quadrula* were studied within the White River and a single population within the Cache River (site C). The Cache River population of *Q. quadrula* possessed 11 monomorphic loci, greater than all of the White River populations combined (10).

Genetic Distance and Identity

Genetic distance (Nei's unbiased) and identity were determined between populations for the four species studied. A genetic dis-

tance of 0.040 ($1 = 0.961$) was determined for the two populations of *A. p. plicata* (Fig. 1). Genetic distance for *P. dombeyanus* ranged from 0.040 to 0.113 ($1 = 0.893$ –0.961). Genetic distance for each of the three populations of *Q. p. pustulosa* ranged from 0.027 to 0.050 ($1 = 0.951$ –0.973). Populations of *Q. quadrula* ranged from 0.012 to 0.084 ($1 = 0.919$ –0.988).

There was a mean genetic distance of 0.333 ± 0.066 ($1 = 0.717$) between the two species of *Quadrula* (Table 2). *Amblyma p. plicata* and *Q. p. pustulosa* had the greatest mean genetic distance of 0.814 ± 0.058 , and *P. dombeyanus* and *Q. p. pustulosa* were the most closely related genera with a genetic distance of 0.396 ± 0.081 . Figure 1 demonstrates the intraspecific and interspecific genetic distances of these species. The cophenetic correlation for the UPGMA phenogram was 0.916, indicating a good fit of the phenogram to the original data matrix.

DISCUSSION

Conspecific Genetic Distance

On the basis of analyzing electrophoretic data from a diversity of taxa, Nei (1978) identified several benchmark criteria for establishing levels of taxonomic differentiation. Populations of vari-

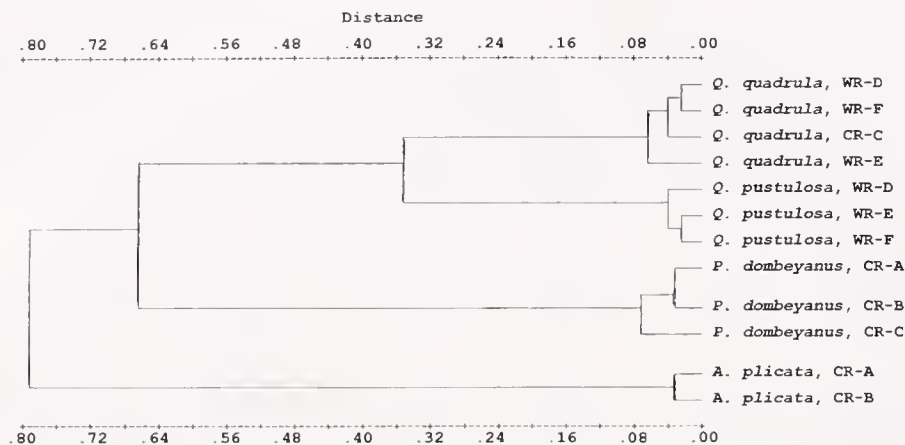


Figure 1. Phenogram showing Nei's unbiased genetic distance (1978) between populations species and genera of *A. plicata*, *P. dombeyanus*, *Q. pustulosa*, and *Q. quadrula* in the White (WR) and Cache River (CR), Arkansas. Cophenetic correlation = 0.916.

ous taxa sharing a genetic identity of 0.90 were typical of conspecifics, somewhat greater than that determined by Davis (1984) for freshwater mussel populations ($I = 0.87$). Conspecific genetic distances of the present study fell within those as determined by Davis (1984) and Nei (1978), with the least genetic similarity for a single population of *P. dombeyanus* (Site C), which exhibited high monomorphism ($I = 0.893$ to Site A). Intraspecific genetic identities for other Unionid mussel species studied are generally high, although values as low as 0.822 ($D = 0.196$) were reported for *Elliptio complanatus* (Lightfoot) conspecifics (Kat and Davis 1984) (Table 3). Berg et al. (1998) identified low genetic divergence between populations of *Q. quadrula* ($\bar{x} = 0.009$; range 0.0005–0.037), even populations separated by 1,550 river miles. These authors concluded that these seven populations studied could be reduced to two interactive metapopulations, and attributed the low distances to high gene flow. Our *Q. quadrula* mussel populations were much closer in geographic distance yet possessed greater genetic distances and significant differences between loci (Johnson et al. 1998).

Gene flow between populations serves to minimize genetic distances between populations (Slatkin 1987), particularly populations of close proximity such as in the present study. Dispersal can occur at the gamete, juvenile, and adult stage of the life cycle to facilitate gene flow. Mussels are sessile animals for which glochidia and the downstream flow of gametes are the possible stages whereby significant migration can occur.

Fish hosts play an important role in the genetic structure of mussel populations by facilitating gene flow. Generalism for host selection may improve opportunities for transformed juvenile to

migrate from one site to another, whereas the tendency for migration of the individual host species during the glochidial attachment phase will alter gene flow. Kat (1983b) found that *Lampsilis* species utilizing anadromous hosts shared greater genetic identities than did species using territorial or strictly freshwater hosts. The flathead catfish (*Pylodictis olivaris* (Rafinesque)) is the sole host identified for the glochidia of *Q. quadrula*, whereas *A. p. plicata* and *Q. p. pustulosa* use a wide range of fish hosts, including the flathead catfish (Howard and Anson 1923, Wilson 1916, Howard 1914, Coker et al. 1921, Stein 1968). No host has been identified for glochidia of *P. dombeyanus* (Oesch 1984). Host diversity may provide the glochidia of *A. p. plicata* and *Q. p. pustulosa* a greater capacity for migrating from one site to another when compared with glochidia of *Q. quadrula* and possibly *P. dombeyanus*. These latter two species had the greatest genetic distances between populations. The flathead catfish has large diurnal migrations (Coon and Dames 1989), which would serve to enhance gene flow for *A. p. plicata*, *Q. p. pustulosa*, and *Q. quadrula*.

Several researchers have correlated migration distance and the capacity for gene flow (Murray and Clarke 1984, Slatkin 1987, Johnson et al. 1988). It was expected for the present study that conspecific populations more distantly separated would exhibit greater genetic distance. This was indeed the case for *P. dombeyanus* and *Q. p. pustulosa*, as upstream and downstream sites exhibited a greater genetic distance than did central sites. However, for *Q. quadrula*, site C of the Cache River shared a greater genetic identity with sites D and F of the White River than did site E, positioned between sites D and F (Fig. 1). Site C of the Cache River is located 71.5 river miles from the nearest White River site. There is no obvious explanation for this anomaly, although similar phenomena were observed by Hornbach et al. (1980) for *Sphaerium*.

Genetic Distance between Species

A congeneric genetic distance for *Quadrula* was determined to be 0.333. This distance is greater than that expected considering that possible hybrids may have been observed for these species (Christian 1995, Posey 1997). It is possible that a third species exists intermediate to both *Quadrula* species studied; this has yet to be investigated. This is a greater genetic distance than congenics of various taxa considered by Nei (1978) {mean $D =$

TABLE 2.

Values of Nei's (1978) genetic identity (above the diagonal) and genetic distance (below the diagonal) for Amblesini in the Cache and White rivers, Arkansas.

Population	<i>A. p.</i>	<i>P. d.</i>	<i>Q. p.</i>	<i>Q. q.</i>
<i>A. p. plicata</i>	***	0.507	0.443	0.455
<i>P. dombeyanus</i>	0.680	***	0.673	0.469
<i>Q. p. pustulosa</i>	0.814	0.396	***	0.717
<i>Q. quadrula</i>	0.787	0.758	0.333	***

TABLE 3.

Mean values of D and standard deviations for pairwise comparisons of conspecific populations within the family Unionidae.

Conspecific Population Comparisons	No. Populations	$\bar{x} \pm S.D.$	Range	Source
Tribe Anodontini				
<i>Anodonta anatina</i> (Linnaeus)	18	0.064 \pm N/A	0.000–0.252	Nagel et al. 1996
<i>A. cataracta</i> (Say)	5	0.034 \pm 0.037	0.001–0.081	Davis et al. 1981
<i>A. cygnea</i> (Linnaeus)	3	0.008 \pm N/A	0.000–0.012	Nagel et al. 1996
<i>Pseudanodonta complanata</i> (Rossmassler)	2	0.000 \pm N/A	N/A	Nagel et al. 1996
Tribe Lampsilini				
<i>Lampsilis cariosa</i> (Say)	3	0.071 \pm 0.021	0.041–0.091	Stiven and Alderman (1992)
<i>L. radiata</i> (Gmelin)	5	0.018 \pm 0.010	0.001–0.033	Kat and Davis (1984)
<i>L. radiata</i> (Gmelin)	3	0.015 \pm 0.005	0.009–0.021	Kat (1983b)
<i>Leptodea ochracea</i> (Say)	2	0.018	N/A	Stiven and Alderman (1992)
Tribe Pleurobemini				
<i>Elliptio complanata</i> (Lightfoot)	15	0.043 \pm 0.025	0.014–0.196	Davis et al. (1981); Kat and Davis (1984)
<i>E. crassidens</i> (Lamarck)	3	0.010 \pm 0.006	0.005–0.018	Davis (1984)
<i>E. icterina</i> (Conrad)	9	0.097 \pm 0.050	0.025–0.184	Davis et al. (1981)
<i>E. mcMichaeli</i> (Clench & Turner)	2	0.014	N/A	Davis (1984)
Tribe Amblesini				
<i>A. p. plicata</i> (Say)	2	0.035	N/A	Present study
<i>P. dombeyanus</i> (Valenciennes)	2	0.074 \pm 0.030	0.040–0.113	Present study
<i>Q. p. pustulosa</i> (L. Lea)	3	0.034 \pm 0.013	0.027–0.050	Present study
<i>Q. quadrula</i> (Rafinesque)	7	0.009 \pm 0.011	0.0005–0.037	Berg et al. (1997)
<i>Q. quadrula</i> (Rafinesque)	4	0.051 \pm 0.030	0.012–0.084	Present study

0.222}, whereas congeneric genetic distances for Unionids range from 0.010 to 1.323 for differing clades (Table 4). Genetic distances should be correlative with time of divergence for genera, as is demonstrated by greater genetic distances for older lineages such as Anodontini and Lampsilini, as compared with lower genetic distances for the more recent Pleurobemini (Davis 1984). These intermediate values obtained for Amblesini are consistent with the geological record.

Tribal genetic distances for the present study ranged from 0.396

for *Quadrula* versus *Plectomerus* to 0.787 for *Amblesini* versus *Quadrula*, with a mean genetic distance of 0.687 ± 0.152 . These intergeneric values are greater than that identified by Mulvey et al. (1997) comparing *Amblesini* and *Megalaniais* (0.516). In comparing studies of other tribes these values are similar to those obtained by Davis (1984) ($\bar{x} = 0.651$; range 0.358–0.935), yet lower than those of Stiven and Alderman (1992) [range 0.825–1.146].

Of particular interest was the genetic relationship of *P. dombeyanus* to *A. p. plicata*. On the basis of immunologic and

TABLE 4.

Mean values of D and standard deviations for pairwise comparisons of congeneric and tribal species within the family Unionidae.

Congeneric Species Comparisons	No. Species	$\bar{x} \pm S.D.$	Range	Source
Tribe Anodontini				
<i>Anodonta</i>	3	0.840 \pm 0.353	0.373–1.323	Kat (1983a)
<i>Anodonta</i>	3	0.501 \pm 0.094	0.417–0.632	Nagel et al. (1996)
Tribe Lampsilini				
<i>Lampsilis</i>	3	0.342 \pm 0.141	0.350–0.420	Stiven and Alderman (1992)
<i>Lampsilis</i>	3	0.958 \pm 0.358	0.213–1.224	Kat (1983b)
Tribe Pleurobemini				
<i>Elliptio</i>	7	0.210 \pm 0.117	0.010–0.446	Davis et al. (1981)
<i>Unio</i>	3	0.308 \pm 0.165	0.216–0.498	Davis (1984)
Tribe Amblesini				
<i>Amblesini</i>	3	0.237 \pm N/A	0.012–0.276	Mulvey et al. (1997)
<i>Quadrula</i>	2	0.360	N/A	Present study
Tribal Comparisons	No. Genera/Species			
Tribe Anodontini	2/4	0.610 \pm 0.052	0.565–0.682	Nagel et al. (1996)
Tribe Lampsilini	2/4	0.962 \pm 0.148	0.726–1.146	Stiven and Alderman (1992)
Tribe Pleurobemini	3/5	0.431 \pm 0.161	0.208–0.725	Davis (1984)
Tribe Amblesini	1/2	0.321	N/A	Davis (1984)
Tribe Amblesini	2/5	0.516 \pm N/A	N/A	Mulvey et al. (1997)
Tribe Amblesini	3/4	0.687 \pm 0.152	0.396–0.814	Present study

morphologic data. Davis and Fuller (1981) suggested that the genera *Amblema*, *Plectomerus*, and *Megaloniaias* were congeneric. This close genetic relationship was not identified by Lydeard et al. (1996) nor in the present study; *P. dombeyanus* was most closely related to *Q. p. pustulosa*. Lydeard et al. (1996) studied the phylogenetic relationships among Unionidae combining sequence data from the 16S rRNA gene and morphologic/reproductive data. They identified a closer relationship of *A. plicata* and *P. dombeyanus* than to *Quadrula*, and questioned the taxonomic utility of the Tribe Ambelmini. Other researchers have identified incongruence between anatomical and genetic relationships among freshwater bivalves (e.g., Hornbach et al. 1980, Davis 1981; Hoeh 1990), yet

good congruence in immunologic and allozyme data (Davis and Fuller 1981). Further evidence of the uncertain taxonomic status of the Unionidae is that Davis and Fuller (1981) found a closer immunologic relationship between *Elliptio* (Tribe Pleurobemini) and *Amblema* than between *Amblema* and *Plectomerus*.

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ZINC ACCUMULATION IN OYSTERS GIVING MOUSE DEATHS IN PARALYTIC SHELLFISH POISONING BIOASSAY

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ABSTRACT During the last 2 years, extracts from cultured oysters (*Ostrea edulis* and *Crassostrea gigas*) intended for screening for paralytic shellfish poisoning (PSP) toxins have on several occasions been lethal to mice upon extended observation periods. The prolonged observation time in the PSP toxins assay was toxins responsible for amnesic shellfish poisoning (ASP). The symptoms observed in the mice deviated from those of both PSP and ASP. Based on lack of knowledge of the toxic principle, and the obligation to protect public health, oyster marketing has been stopped on such occasions. The probable solution of the problem was suggested to us by oyster farmers who, via international contacts, brought to our attention similar Canadian experiences. They found that occasionally oysters accumulate large amounts of zinc which are lethal to mice when amounts injected intraperitoneally exceed about 450 µg in a 20 g mouse. When analyzing zinc levels in the suspect Norwegian oyster extracts, correlation with the Canadian data was very convincing, indicating the same "false positive" result in our analyses. Zinc at this level presents no health threat to humans with normal consumption of oysters. This article is written as a caution to other regulatory personnel involved in oyster monitoring against installing unnecessary restraints on oyster farmers before considering the possibility of zinc giving "false positive" results.

KEY WORDS: Oysters, zinc, PSP, false positives

INTRODUCTION

Mussels and oysters in Norway are routinely analyzed for the presence of algal toxins before they legally can be marketed. The monitoring program consists of methods for quantification of toxins contributing to diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP), and amnesic shellfish poisoning (ASP). Though the two former intoxications have been known for many years, ASP was reported for the first time following an incident in Canada in 1987. ASP is caused by an amino acid called domoic acid. Presence of domoic acid in mussels can be detected when injecting the PSP extracts intraperitoneally (i.p.) in mice. Symptoms of ASP appear in 3–4 h, and consequently the mice are observed for several hours. During the last 2 years, extracts from cultured oysters (mainly the Norwegian flat oyster *Ostrea edulis*, but occasionally *Crassostrea gigas* originally from the Pacific) tested for PSP/ASP toxins in mice have given unusual symptoms, not associated with either PSP or ASP, leading to mouse death within a few h. The reason for this unknown toxicity was obscure since the mouse lethality could not be explained by any known marine algal toxins nor microorganism, and it resulted in problems for the oyster farmers. Experiences of an oyster farmer led to a suggestion of a solution to the problem through a request via the international data network. Canadian scientists experienced similar problems which they found were due to specific accumulation of

zinc in oysters. With this background information, analyses of zinc and several other metals in oysters were performed in an attempt to find the compound responsible for the unknown toxicity.

MATERIALS AND METHODS

Oysters (*Ostrea edulis* and *Crassostrea gigas*), as well as mussels, are sent to the Norwegian College of Veterinary Medicine for detection of toxin before they can be marketed. The analyses are routinely aimed at screening samples for DSP, PSP, and more recently, ASP toxins.

Extraction for PSP (and ASP) Toxins

The extraction is performed according to the AOAC method for PSP toxins (1995). Wet oyster tissue is homogenized and extracted in 0.1 N HCl, giving a final concentration of 100 g oyster tissue in 200 mL, and with a pH adjusted to 3 ± 0.5 .

Mouse Bioassay

Mice weighing between 15 and 20 g each are injected intraperitoneally with 1 mL of the extracts, and symptoms and survival times are registered.

TABLE 1.

Results from routine screening of oysters (*Ostrea edulis* and *Crassostrea gigas*) in 1996/97.^a

Sample Site	Date of Sampling	Survival Time	Symptoms ^b
Bjerga Osters, <i>O. edulis</i>	96-10-22	2 h 30 min/4–18 h	Intermediate
Bjerga Osters, <i>O. edulis</i>	96-12-03	1 h 45 min/4–18 h	Strong/intermediate
Bjerga Osters, <i>O. edulis</i>	97-02-11	4–18 h	Intermediate
Bjerga Osters, <i>O. edulis</i>	97-02-21	2 h 30 min	Intermediate
Sealife, <i>C. gigas</i>	96-08-06	>24 h	None
Sealife, <i>C. gigas</i>	97-04-29	4–18 h	Intermediate
Sealife, <i>C. gigas</i>	97-06-10	>24 h	None
Sealife, <i>C. gigas</i>	97-10-07	4–18 h	Intermediate
Sealife, <i>C. gigas</i>	97-11-04	2–4 h	Strong
Sealife, <i>C. gigas</i>	97-12-01	6–8 h/>24 h	Intermediate/weak
Aga musling, <i>O. edulis</i>	97-11-30	>24 h	None
Aga musling, <i>O. edulis</i>	97-12-09	30 min	Strong
Aarefjord, <i>O. edulis</i>	97-11-30	4–18 h/>24 h	Weak
Taroskjell, <i>O. edulis</i>	97-12-10	30 min	Strong

^a (Two mice for each sample), i.p. injections in mice of 1 mL extracts representing 0.5 g mussel meat.^b The symptoms showed escalating differences in severity; when the mice died within a few hours, the symptoms were marked, dominated by the mice lying down, turning on their sides, breathing heavily, paralysis of the hind legs, stretching of the hind legs, immobilization, and finally respiratory arrest. When the mice survived more than 2 h, the symptoms was less severe, and paralysis of the hind legs was less obvious. Some of the mice surviving 24 h showed symptoms of being affected (sick, reduced mobility), but no paralysis of the hind legs was observed.

Pathological Examinations

A necropsy was performed immediately following death of the mice. Sections from the brain, lungs, heart, liver, kidneys, and intestines were fixed in 10% phosphate-buffered formalin, processed routinely, and stained with hematoxylin and eosin.

Metal Analyses

The oyster extracts were surveyed for several metals at the National Veterinary Institute. Extracts for mercury were wet digested with nitric acid 65% (w/v) and perchloric acid 70% (w/v), and all other extracts were diluted with 15% v/v concentrated HNO₃ before analysis. All elements were determined by atomic absorption spectroscopy (AAS) (Vandecasteele and Block, 1993) with external calibration and deuterium background correction (Varian Spectr AA 400). Mercury was analyzed by cold-vapor AAS (CVAAS). Aluminum, cadmium, and lead were analyzed by electrothermal graphite furnace AAS-technique (ETAAS) with pyrolytical platforms. A mixed modifier of palladium and magnesium nitrate was used for the determination of aluminum and cadmium, and a modifier of palladium and ammonium dihydrogenphosphate was used for the determination of lead. Copper and zinc were analyzed by flame AAS-technique (FAAS) with lean flame of acetylene/air. The recovery varied between 105 and 120%, and the reproducibility between 5 and 10%. The detection limits for the elements (in µg/L) were as follows: Al (0.7), Cd (0.04), Pb (0.42), Hg (2.24), Cu (30), and Zn (7).

RESULTS

Table 1 shows the results from the analyses of oysters performed during 1996 and 1997. As can be seen samples collected in the winter periods were all toxic with one exception, whereas the few samples collected during the summer period were nontoxic.

Analyses of several essential as well as nonessential metals in selected oyster samples from Table 1 were performed, as shown in Table 2. Metal analyses were carried out on toxic as well as nontoxic samples of oysters.

Results from the analyses of zinc are shown in Table 3 permitting estimation of correlation between zinc levels and i.p. toxicity in mice. The table shows that lethal effects of oyster extracts correspond with zinc levels of about 400 µg/20 g mouse. Based on short survival time and geographic location, the oyster extract from Aga (97-12-09) was selected for studies of dose-related toxicity, including pathological studies. According to the results shown in Table 4, lethal effects seem to be associated with i.p. injection of samples containing zinc above approximately 400 µg/20 g mouse.

No organ changes were demonstrated upon gross and histopathological examinations. The oysters from Aga (97-12-09) were selected for dose-response studies based on short survival time in the routine assay, before results from the zinc analyses were known. Since the zinc level was relatively modest in this sample, another oyster sample, from Bjerga 97-02-21, was also tested in a dose-response manner (Table 5). From the Table one can see that acute toxicity of the oyster extract is dose dependent, and lethal effects correspond with zinc levels of about 400 µg/20 g mouse.

DISCUSSION

The problem was recognized when the mice injected with oyster extracts according to the method for PSP (and ASP) toxins displayed symptoms deviating from those of PSP and ASP when

TABLE 2.

Result of metal analyses of selected oyster samples from Table 1.^a

Sample	Date	Al	Cd	Cu	Hg	Pb	Zn
Bjerga, <i>O. edulis</i>	97-02-21	1.10	1.10	8.80	0.06	0.04	1,580
Sealife, <i>C. gigas</i>	97-06-10	0.54	0.38	4.40	0.06	0.06	240
Sealife, <i>C. gigas</i>	97-12-01	0.78	0.56	13.60	0.06	0.12	600
Aga, <i>O. edulis</i>	97-12-09	0.98	1.40	9.00	0.14	0.10	820
Taroskjell, <i>O. edulis</i>	97-12-10	1.06	1.26	9.60	0.10	0.04	1,540

^a Results in mg/kg wet weight.

TABLE 3.

Association between zinc level injected i.p. in mice, adjusted to 20 g body weight (as in the PSP mouse bioassay) and survival time (two mice) for each sample.

Sample, Zn-Conc.	Date of Sampling	Zn, µg/20 g b.w.	Survival Time
Bjerga	97-02-21	1,050	2 h 30 min
790 µg/mL		1,056	2 h 30 min
Sealife	97-06-10	150	>24 h
120 µg/mL		160	>24 h
Sealife	97-12-01	400	>24 h
300 µg/mL		375	6-8 h
Aga	97-12-09	482	30 min
410 µg/mL		497	30 min
Taroskjell	97.12.10	963	30 min
770 µg/mL		1,027	30 min

the mice were observed for several hours. When PSP toxins are present, the mice die within a short time (minutes) with severe cramps. Death time is determined as time elapsed from completion of injection to last gasping breath (AOAC 1995). The reactions upon i.p. injection of ASP toxin, on the other hand, are characterized by involuntary scratching of both shoulders with the hind legs typically within 7–21 minutes upon injection (Todd 1993). Movements become increasingly uncoordinated, the mice fall on their sides and eventually die. In the case with extracts of cultured Norwegian oyster (*Ostrea edulis*) sampled at different locations and *Crassostrea gigas* from one location, the mice showed general weakness, fell on the side, displayed paralysis and stretching of the hind legs, and eventually died without any signs of cramps. Death times varied between 30 min and 18 h with the different extracts. When blue mussels (*Mytilus edulis*) from the same location as one of the positive oyster extract (Bjerga Osters) were analyzed no toxicity in the mice was observed (data not shown). This indicated a specific toxic factor associated with oysters. Presence of micro-

TABLE 4.

Toxicity in mice injected (i.p.) with 1 mL of stepwise dilutions of oyster (*Ostrea edulis*) extract from Aga (97-12-09).^a

Zn Conc. of Extracts	Mouse No.	µg Zn/20 g b.w.	Survival, Min	Symptoms
410 µg/mL	1	512.5	35	Strong symptoms,
410 µg/mL	2	546.7	21	paralysis of hind legs,
410 µg/mL	3	497.0	32	gasping before death
273 µg/mL	1	321.2	>210	General weakness,
273 µg/mL	2	341.3	>210	slow motions, legs
273 µg/mL	3	376.6	>210	backwards
205 µg/mL	1	273.3	>210	No symptoms
205 µg/mL	2	282.8	>210	
205 µg/mL	3	264.6	>210	
137 µg/mL	1	182.7	>210	No symptoms
137 µg/mL	2	166.0	>210	
137 µg/mL	3	176.8	>210	
0.1N HCl	1		>210	No symptoms
0.1N HCl	2		>210	
0.1N HCl	3		>210	

^a Doses were calculated per 20 g body weight which is standard for the PSP assay (three mice/dose).

TABLE 5.

Toxicity in mice upon injections (i.p.) with stepwise dilutions of oyster (*Ostrea edulis*) extract from Bjerga (97-02-21).^a

Zn-Conc. of Sample	Mouse No.	µg Zn/20 g b.w.	Survival Time	Symptoms
790 µg/mL	1	930	2 h 5 min	Weakness, lays on side,
790 µg/mL	2	1,000	2 h	paralyzed hindlegs
395 µg/mL	1	552	8–18 h	Weak, partly paralyzed
395 µg/mL	2	510	>24 h	hindlegs, one recovered
198 µg/mL	1	258	>24 h	None

^a Doses were adjusted to 20 g body weight.

organisms is routinely screened, and oyster samples giving unusual responses in the mouse bioassay were all undetectable for toxins from microorganisms.

Since the toxic factor in oysters towards mice was unknown, marketing of the oysters was prohibited in order to protect consumers from any possible health risk. The oyster farmers protested when this problem seemed to repeat itself in the winter seasons of 1996 and 1997. One of the farmers informed us that he had consumed two dozen oysters once weekly for four weeks during November/December 1997, during a period when his oysters were banned from consumption. According to him, he had felt no ill effects.

During late fall of 1997 another oyster farmer received information from a Canadian group of researchers who had experienced quite similar problems some years ago (McCulloch et al. 1989). They concluded that periodically oysters accumulate high concentrations of zinc, which are lethal to mice upon i.p. injection at levels exceeding about 450 µg/20 g mouse and corresponds to 900 µg Zn/100 g oyster meat. We selected both positive and negative oyster samples for screening of several metals (Table 1). As can be seen from Table 2, among the metals analyzed, only zinc displayed concentrations in some of the samples which can be associated with toxicity in mice. In Table 3, the zinc levels injected in the mice (weighing between 15 and 17 g) are calculated at 20 g body weight, facilitating comparison with the Canadian data. As can be seen, lethal effects were experienced when zinc levels exceeded about 400 µg/20 g mouse. The only result that deviated was the apparent high toxicity of the sample from Aga, compared with the relatively low zinc level.

Before the results of the metal analyses were known, a dose-response study of dilutions of one of the extracts giving shortest survival time, from Aga, was performed (Table 4). As can be seen from the table, toxicity decreased with the dilution of zinc. However, the survival time following injections of the extract from Aga was surprisingly short compared with the other toxic samples. The reason for this seemingly high toxic potential is unknown, and contribution by other unidentified toxins cannot be ruled out. The mice from this experiment were pathologically examined as well, however, no pathological changes were observed.

When results from the metal analyses were known, another sample, from Bjerga, was also tested in a dose-response manner towards mice (Table 5). Again, the lethal effects corresponded well with the zinc concentrations, and the symptoms fit those described by the Canadian group (McCulloch et al. 1989).

Based on the results from Canada, our results with oysters collected at different times of the year, at different locations, strongly indicate that elevated levels of zinc are the reason for the

toxicity in mice. When assessing possible human toxicity it is important to consider oral versus intraperitoneal toxicity. Generally, toxic compounds are between one and two orders of magnitude less acutely toxic via the oral route compared with i.p. injections. In the case of zinc, we have no data on oral toxicity in mice, but in rats the LD₅₀ from acute oral toxicity of inorganic zinc solutions is about 2.5 g/kg body weight. According to McCulloch et al. (1989), toxicity of a zinc-chloride solution is somewhat lower than the toxicity of zinc in mussels, having a lethal effect upon i.p. injections of about 600 µg/20 g mouse, corresponding to 30 mg/kg body weight. Zinc is not considered very toxic to man when ingested via the gastrointestinal tract, and even a level of about 1,000 µg/100 g of oyster meat does not present a health problem for consumers (Brown and McPherson 1992). The intentionally high consumption of oysters by the aforementioned oyster farmer indicates the same.

Hiltner and Wichmann (1919) had already found high zinc levels in oysters (*Ostrea edulis*). Others (Gaarder and Alvsaker 1941, Julshamn 1981a) have confirmed those zinc values. The authors reported values of 4 g Zn/kg dry matter in whole oyster (*O. edulis*) sampled at the same place on the Western coast of Norway

(Innerøy). The results indicate that the zinc content in water has not increased during the 40-y span between the two investigations. Norwegian flat oysters (*Ostrea edulis*) show natural high zinc levels and higher levels than Pacific oyster (*Crassostrea gigas*) and American oyster (*Crassostrea virginica*) (Pringle et al. 1968). This seems to correspond with our data, although the number of analyses performed is small. In a study on seasonal variations in the content of elements in oysters (*Ostrea edulis*), the zinc content of immature oyster tissue showed the highest values in April, May, and August (Julshamn 1981b). Zinc content also changes during maturation and spawning.

This has been an important lesson for us: When unusual results are obtained, both a literature survey and requests via international data systems may prove helpful. The problem with literature searches are that you do not know what to ask for, whereas a general request to international experts might reach somebody with similar experience.

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EXAMINATION OF LEAD LEVELS IN THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*, FROM THREE SITES IN MAINE

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ABSTRACT Bioaccumulation of lead in marine organisms has been a concern of researchers, government, and other food safety. The feeding habits of the American Lobster, *Homarus Americanus*, prior to recruitment into the fishery, gives rise to its potential accumulator of environmental contaminants. Three sites indicative of common lobster fishing areas were selected along the coast of Maine to assess and establish baseline level for lead contamination. From each of these sites, lobsters averaging in weight between 450 and 550 g, were collected and samples were taken from the gills, meats (mixture of tail and claw meat), and the hepatopancreas to be analyzed for lead concentration. Lead levels ranging from 20 to 101 ppb were found. Levels were higher in the hepatopancreas and meats than in the gills. There were interactions between location and body portion resulting from the higher levels in the hepatopancreas and meats. The levels found were significantly below the FDA/EPA limits on edible portions.

KEY WORDS: Lead, bioaccumulation, American Lobster, environmental contamination

INTRODUCTION

Environmental contamination by heavy metals such as lead (Pb) is generally reflected by an increase in the tissues of aquatic animals. Several investigations by researchers (Naqvi and Howell 1993a,b; du Preez et al. 1993; Pastor et al. 1994) have shown that crustaceans are bioaccumulators of heavy metals like lead and as such are indicators of environmental contamination. Naqvi and Howell (1993a) studied the uptake of cadmium and lead in Red Swamp Crayfish and found that these metals accumulate in the exoskeleton, hepatopancreas, gills, mid-gut glands, and abdominal muscles of crayfish. They found that uptake was timedependent and crayfish rapidly accumulate these metals, where they remained in tissues for a long period of time. In another study using Red Swamp crayfish, Naqvi and Howell (1993b) showed that lead has detrimental effects on the reproduction of the crayfish and subsequently on the crayfish farming industry. Lead contamination would have similar detrimental effects on the lobster industry of Maine. The freshwater crab, *Potamonautes warreni*, was used to study the accumulation of zinc and lead by du Preez et al. (1993). They found the level of lead was approximately 87 parts per million (ppm) in the gonads and carapace tissues. The research made no conclusion concerning the use of freshwater crabs as biomonitors. Pastor et al. (1994) analyzed several marine organisms (fish, mollusks, and crustaceans) for heavy metals (cadmium, lead, and mercury). They found a wide range of lead concentrations, from 53–2220 parts per billion (ppb), in the various organisms. Pastor et al. concluded that mollusks are useful bioindicators for cadmium and lead.

The American lobster (*Homarus Americanus*) is caught and used largely for human consumption. Concern has been raised about the presence of lead in these crustaceans, and the concern over lead contamination is important to the long-term survival of the lobster fishery and the human consumption of harvested lobsters. The American lobster is carnivorous, a bottom feeder, and as such is likely to bioconcentrate heavy metals if these metals exist in the surrounding environment. Since lobsters exhibit limited movement until they are at the size/age recruited to the fishery, they become a representative of their environment (Daniel et al. 1989). In a study of trace elements in a fishery resource, Hall et al.

(1978) reported lead levels of 500–600 in a mixture of claw and tail meats of the American lobster. In a calibration study for trace metals in biological tissues, Berman and Boyko (1986) reported an average of 5.57 (± 1.70 SD) ppm lead in the lobster hepatopancreas. Peterson and Mortensen (1994) found a range of 0.031–0.070 ppm of lead in lobsters on the Danish national markets. In a Maine Department of Environmental Protection (MeDEP) report, Sowles et al. (1996) detected levels of 1.31 (± 0.44 SD) ppm lead in the meats (mixture of tail and claw meats) of lobsters and 0.66 (± 14 SD) in the hepatopancreas. Though these data are varied and cover a wide range of areas, they also indicate a need to quantify lead levels in lobsters in further detail. The purpose of this study was threefold: to sample the lobster fishery along the coast of Maine in response to lead consumption concerns, establish baseline levels for lead in Maine lobsters, and compare those levels with the FDA limits for concern in human consumption.

MATERIALS AND METHODS

At each of three sampling locations along the coast of Maine (Bremen, Pine Point, and Stonington, Maine), 18 commercially harvested lobsters were collected. Figure 1 gives the locations of the sampling sites along the Maine coast. These sites were selected because they are major lobster buying points and as such are fairly representative of the lobster fishery in Maine. These sites also represent three main areas of interest for potential contamination and are major lobster fishing areas. Pine Point is in the southern Maine coastal area near Casco Bay, a high population center, and therefore considered not to be a clean region; Bremen is in the mid-coast region of Maine and a large lobster buying port; and Stonington is the beginning of the downeast section of the Maine coast. The lobsters harvested for this study were of legal size, ranging in weight from 455 to 567 gs. The lobsters were stored in plastic bags on ice during transport to the University of Maine analytical laboratory. Body portion samples from the gill, tail meat, and hepatopancreas of each of the lobsters were taken and used for laboratory analysis. The samples were excised and prepared for lead analysis.

Low temperature dry ash method (Moyse and Fernandez 1987)

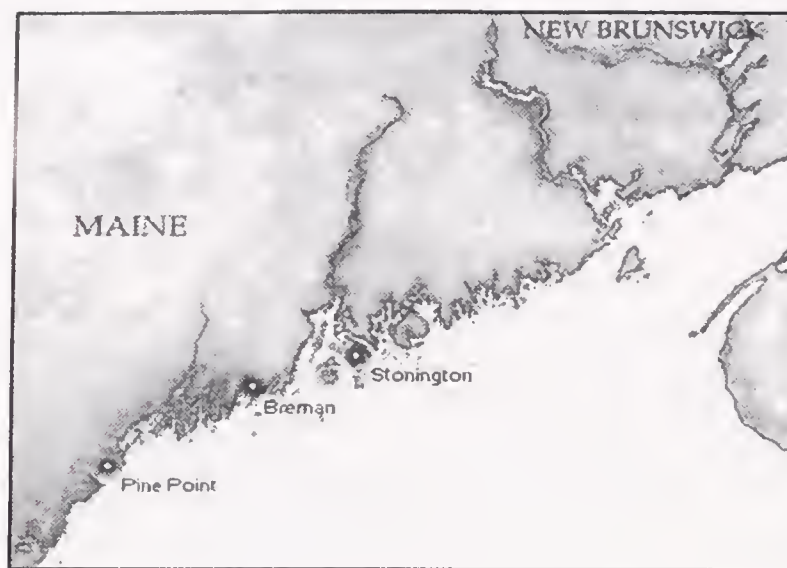


Figure 1. Sampling site locations along the coast of Maine.

was used to test for lead contamination. Samples were placed in crucibles, cleaned with dilute nitric acid, and dried in a non-force-draft oven and dry-ashed at 425°C. After ashing, the samples were digested in a nitric acid and hydrogen peroxide mixture as per the standard method. A graphite furnace (model 188, Thermo Jarrell Ash, Franklin, MA) and atomic absorption spectrophotometer (model Video 12, Instrumentation Lab, Franklin, MA) were used to assess the samples for detection of lead levels. Samples were analyzed individually and care was taken to prevent cross-contamination. Following the normal lead analysis procedure of the university analytical laboratory, all samples were run in triplicate and reagent blanks were analyzed. The reagent blank values were subtracted from the sample values and the result is reported herein. All data reported in the results were above detection limits of the methods and instruments used.

The results from the laboratory analysis were analyzed using the analysis of variance procedure, general linear models (GLM), of the Statistical Analysis System (SAS). These analyses and the means separation tests were performed using the Duncan's Multiple Range Test (SAS 1990).

RESULTS AND DISCUSSION

Table 1 summarizes the lead level data in ppb showing average and standard deviation by location and body portion. Total body lead level reported is the mathematical average of the gill, meat, and hepatopancreas levels. The general trend of the data suggests that the lowest levels of lead are found on the gill followed by the

tail meat and then the hepatopancreas. This trend is followed with the exception of the Stonington location where, overall, it exhibits greater variation in the data.

The results exhibit high SDs so a two-way analysis of variance (ANOVA) was performed to resolve sources of variation. ANOVA revealed significance in the body portion and the interaction of location and body portion. As seen in Table 1, there is a positive correlation between lead level and body portion at the various locations. Physiologically, it is expected that lead would be concentrated in the hepatopancreas as it serves the same filtration function as does the liver in humans. Lower values are seen in the gill area as expected. These trends are shown in Tables 1 and 2. Also, there is high variability within location that contributes significantly to the model. The analysis reveals significance ($p \leq .05$) between location and body portion. Lobsters from the Stonington location had higher concentrations of lead in the tail body portion. This interaction may be an artifact of high variation between lobster and within location variation. No site-specific or analysis-specific data collected contribute to the reason. There was no positive correlation between lead level values in one body portion to values in other body portions within the lobster.

The means separation data are given in Table 2. In the means separation using the Duncan's New Range Test, no difference was found between locations. The means separation on body portion yielded two significantly different groups of means; the higher group included the tail and hepatopancreas means and the lower group the gill mean. Because of the large variation in this study, the tail and hepatopancreas means were not significantly different. These results contradict a previous study by Sowles et al. (1996)

TABLE 1.

Average and standard deviation (\pm SD) lead levels for American lobster, *Homarus Americanus*, given in ppb by location and body portion sampled.

Location	Body portion		
	Gill	Meats	Hepatopancreas
Bremen	25.67 (\pm 67.96)	61.07 (\pm 6.72)	93.72 (\pm 6.81)
Pine Point	17.89 (\pm 16.25)	76.95 (\pm 6.87)	100.22 (\pm 103.30)
Stonington	20.97 (\pm 24.07)	161.59 (\pm 153.56)	51.00 (\pm 33.39)

TABLE 2.

Means separation performed by Duncan's Multiple Range Test. Means with different letters are significantly different.

Location	Mean (ppb)	Body Portion ¹	Mean (ppb)
Bremen	60.14 a	Gill	21.51 a
Pine Point	64.56 a	Hepatopancreas	82.23 b
Stonington	76.67 a	Tail	100.23 b

¹ There is a significant interaction of location and body portion.

that proposes a preliminary relationship of (hepatopancreas lead level concentration) = $0.50 \times$ (meat lead level concentration). As stated previously, it is expected that the gill would be the lowest in lead concentration. Since lead would concentrate in the body mostly due to ingestion, the amount of lead found in the gills would represent a fraction of the lead that was brought in through the gills during the process of ventilation and secretion as opposed to being ingested. Because of concentration, the meats and hepato-

pancreas portion lead levels are more indicative of the amount of lead in the lobster available for human consumption.

The locations selected in this study were representative of lobster fishing areas along the entire coast of Maine. The results of this study are well below those found by other researchers previously discussed and establish good baseline data. Lead levels do not appear to be a public health issue in the consumption of Maine lobster.

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EFFECT OF AN ORGANOPHOSPHORUS INSECTICIDE, FENITROTHION, ON SURVIVAL, OSMOREGULATION, AND ACETYLCHOLINESTERASE ACTIVITY IN DIFFERENT LIFE STAGES OF TWO PENAEID SHRIMPS: *PENAEUS STYLIROSTRIS* AND *PENAEUS VANNAMEI* (CRUSTACEA, DECAPODA)

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ABSTRACT The acute toxicity of fenitrothion was studied in seawater (36 PSU = 1060 ± 10 mosmkg⁻¹) in larval, postlarval, and juvenile instars of the shrimp *Penaeus stylirostris* and *Penaeus vannamei*. The effects of this organophosphorus insecticide on juvenile hypo-osmoregulatory capacity (hypo-OC), i.e., the difference between the osmolality of the haemolymph and that of seawater) and muscle acetylcholinesterase (AChE) activity were then recorded for both species at lethal and sublethal concentrations. In *P. stylirostris*, 24 and 48 h LC₅₀s for nauplii were above 500 µg l⁻¹. The 24-h LC₅₀s ranged from 160 µg l⁻¹ (zoae 2) to 12 and 10 µg l⁻¹ (mysis 3 and postlarval instar 1) and from 11.2 µg l⁻¹ (juveniles of 8 g) to 18.9 µg l⁻¹ (juveniles of 20 g). The 48-h LC₅₀s ranged from 42 µg l⁻¹ (zoae 1) to 2 µg l⁻¹ (mysis 3) and from 10.5 µg l⁻¹ (juveniles of 8 g) to 16.3 µg l⁻¹ (juveniles of 14 g). In *P. vannamei*, 24 and 48 h LC₅₀s for nauplii were above 500 µg l⁻¹. The 24 h LC₅₀s ranged from 324 µg l⁻¹ (zoae 1) to 12 and 9 µg l⁻¹ (mysis 3 and postlarval instar 1) and from 19.2 µg l⁻¹ (juveniles of 7 g) to 41.5 µg l⁻¹ (juveniles of 18 g). The 48 h LC₅₀s ranged from 29 µg l⁻¹ (zoae 2) to 10 and 8 µg l⁻¹ (mysis 3 and postlarval instar 1) and from 19.2 µg l⁻¹ (juveniles of 8 g) to 28.7 µg l⁻¹ (juveniles of 12 g). Thus, for both species, tolerance to fenitrothion in seawater decreased with the larval development but increased with the weight of the juveniles. *P. vannamei* was more tolerant to fenitrothion than *P. stylirostris*, at most stages. In *P. stylirostris*, fenitrothion decreased hypo-OC and AChE activity. The decrease in hypo-OC was dose and time dependent. After 24 h of exposure at different sublethal fenitrothion concentrations, hypo-OC and AChE activity decreased significantly by 5–22% and by 13–18%, respectively. In *P. vannamei*, hypo-OC and AChE activity did not vary after exposure to sublethal concentrations. Only juveniles exposed to lethal concentrations and moribund shrimps showed significant AChE activity decreases. Hypo-OC and AChE activity appeared as good indicators of physiological disturbance because of the pesticide in *P. stylirostris* but not in *P. vannamei*.

KEY WORDS: Acetylcholinesterase, crustaceans, fenitrothion, organophosphorus insecticide, osmoregulation, penaeids

INTRODUCTION

Fenitrothion [0,0-dimethyl 0-(3-methyl-4-nitrophenol) phosphorothioate] is one of the most effective organophosphorus (OP) insecticides and is widely used because of its rapid degradation in the environment and its relatively low toxicity to mammals. It represents a potential threat for nontarget species such as freshwater and marine invertebrates (Bhagyalakshmi and Ramamurthi, 1980; Tronczynski, 1990; Lignot et al., 1997). Fenitrothion can contaminate the aquatic environment through drift of aerial spray, either during crop spraying or during mosquito killing programs, watershed drainage, and/or accidental spillage (Clark et al., 1987).

Crustacean aquaculture is mainly based on the culture of penaeid shrimps (Lee and Wickins, 1992), and is conducted in coastal areas potentially subject to pollutants in general, and fenitrothion in particular.

Fenitrothion, like other OP insecticides, is a potent inhibitor of acetylcholinesterase (AChE) activity, an enzyme essential to the correct transmission of nerve impulses (Fukuto 1990; Kennedy, 1991). OP insecticides act as nerve poisons by blocking synaptic transmission in the cholinergic parts of the nervous system, i.e., by inhibiting the activity of AChE. The reduction in or inhibition of this enzyme activity has been used successfully in combination with other measurements (e.g., chemical residues, behavior and/or toxic response) as a tool in the diagnosis of organophosphate poi-

soning in fish (Lockhart et al., 1985; Zinkl et al., 1987) and aquatic invertebrates (Macek et al., 1972; Day and Scott, 1990).

Although data on the inhibition of crustacean AChE activity by OP insecticides are abundant (Coppage 1974; Schoor and Brausch, 1980; Reddy and Rao, 1988; Repetto et al., 1988; Morgan and Kiceniuk, 1990; Reddy et al., 1990; Bocquené and Galgani, 1991; Gälli et al., 1994), little direct attention has been focused on the effect of these insecticides on osmoregulation (Péqueux, 1995). The ability to osmoregulate (osmoregulatory capacity) has been proposed, however, as an indicator of the physiological condition of crustaceans, particularly penaeid shrimps (Charmantier et al., 1989). It has proved sensitive to a wide array of adverse conditions, such as turbidity (Lin et al., 1992), low pH (Allan and Maguire, 1992), low oxygen concentrations (Charmantier et al., 1994), presence of metals (Bambang et al., 1995a, Bambang et al., 1995b), ammonia (Young-Lai et al., 1991; Lin et al., 1991, 1993; Chen and Cheng, 1996) in the medium and, recently, insecticide and tributyltin oxide exposures (Lignot et al., 1997, 1998).

The objectives of this research were to (1) evaluate the acute toxicity of fenitrothion to the penaeid shrimps *Penaeus stylirostris* and *Penaeus vannamei* for larval, postlarval, and juvenile instars by determining the 24 and 48 h median lethal concentrations (LC₅₀s); (2) determine the hypo-osmoregulatory capacities and muscle acetylcholinesterase activities of animals subjected to lethal and sublethal concentrations of fenitrothion; and (3) determine

whether such measurements are useful diagnostic tools to detect sublethal toxicity of low concentrations of fenitrothion.

MATERIALS AND METHODS

Animals

Larval and postlarval instars were collected from the hatchery of AQUACOP (Ifremer research center in Tahiti, French Polynesia) and were used on site. The developmental stages were determined by microscopic examination according to the method of Hudnaga (1942).

Juveniles were collected at the AQUACOP center from 500 m² earthen ponds and were transferred to the on-site laboratory and kept in tanks for 3 days ($S = 36 \pm 1$ PSU, $T = 27 \pm 1^\circ\text{C}$, 12 L/12 D photoperiod). Each tank contained 2 m³ of filtered recirculated seawater. Juveniles were fed FUC® dry pellets (composition: 40% of crude protein, 8% of lipids, and 12% of ash) (Cuzon, pers. comm.) during the first 2 days of the acclimation period. Animal wet weights ranged from 8 to 20 g for *P. stylirostris* and from 7 to 18 g for *P. vannamei*. Intermolt stages of juveniles were determined by microscopic examination of antennal scales according to the method of Drach and Tchernigovtzeff (1967).

Acute Toxicity of Fenitrothion

Acute toxicity tests were used to estimate the fenitrothion concentration, expressed as the LC₅₀, which is the lethal concentration to 50% of the test organisms in the time period prescribed by the test. Experimental conditions for the LC₅₀ determinations were based on EPA recommendations (Rieder, 1985; Klemm et al., 1990). Acute tests with water renewal every 24 h were used. LC₅₀s and 95% confidence intervals were determined for larval and juvenile stages after 24 and 48 h of exposure. LC₅₀ and 95% confidence intervals were calculated with a computer program based on the probit model described by Finney (1962) and Zitzko (1982) and adapted by R. Mounet-Guillaume (unpublished). Twenty animals per concentration were used and were divided into groups of 10 shrimps per tank.

Larval toxicity tests were acute static tests conducted in Petri dishes containing 40 ml of seawater (SW). Passive diffusion through the surface allowed aeration of the water samples. Nauplii at mixed stage III–V were used. Zoeae I–III and mysis I–III at the beginning of their stages were retained. During exposure, nauplii were not fed, zoeae were fed *Phaeodactylum tricornutum*, and mysis were fed *Artemia* nauplii. Postlarval acute toxicity tests were conducted in plastic boxes containing 250 ml of SW. Continuous aeration and food (adult *Artemia* sp.) were provided. For juveniles, plastic tanks containing 40 L of SW were used. Continuous aeration was provided. Only juveniles in intermolt stage C were retained. Juveniles were not fed during exposures. For larval, postlarval, and juvenile instars, experiments were conducted in seawater at 36 ± 1 PSU and $27 \pm 1^\circ\text{C}$ with a 12 L/12 D photoperiod.

Test solutions were prepared using successive dilutions from a fenitrothion stock solution containing 500 µg fenitrothion.l⁻¹ The stock solution was prepared by dissolving reagent-grade fenitrothion (99% purity) from Bayer AG in technical grade acetone. For each fenitrothion toxicity test, a control group was exposed to a quantity of acetone equivalent to the maximum amount of test solution used in each test (50 µl for larval and postlarval instars and 500 µl for juvenile instars). Only the results of those tests where the acetone test group mortality was less than 10% were

retained. The absence of body movement and immobility of heart and scaphognathite after repeated touches with a probe were considered as proof of mortality. Regular observations were made and dead individuals were removed 3, 6, 12, 24, and 48 h after the beginning of the tests.

In order to determine the stability of the fenitrothion concentrations in the test media, 500 ml SW samples were collected after 0, 24, and 48 h of contamination, and fenitrothion was titrated on a Hewlett Packard gas chromatograph (internal column diameter 2 mm, oven temperature 175°C) according to the method developed by Mestres et al. (1969). Samples were extracted using equal quantities of ethyl ether and petroleum ether. Extracts were evaporated up to 1 ml and directly injected into the gas chromatography equipment. Initial concentrations of 1, 2, 4, and 20 µg.l⁻¹ were used. The recovery rate of fenitrothion during gas chromatography titrations was 90%.

Effect of Fenitrothion on Osmoregulatory Capacity

Hypo-osmoregulatory capacity (hypo-OC) was determined in juveniles by establishing the difference between their hemolymph osmolality and the osmolality of the external medium (Charman-tier et al., 1989). These osmolalities expressed in mosm.kg⁻¹ (100 mosm.kg⁻¹ \approx 3.4 PSU) were measured on a Wescor 5500 osmometer requiring 10 µl samples. The effect of fenitrothion was evaluated by determining hypo-OC in full-strength SW at 1060 ± 10 mosm.kg⁻¹ (\approx 36 PSU) after a given period of exposure to the insecticide. Hemolymph was sampled from juveniles by inserting the needle of a 1-ml hypodermic syringe into the ventro-lateral sinus of the abdomen. The wet weight of the shrimps was determined after each determination. Only animals in intermolt stage C were retained for the titrations.

In *P. stylirostris*, the effect of time was measured for one sublethal concentration (10 µg.l⁻¹) over 12 h (experiment 1: short-term time effect). The effect of the concentration of fenitrothion was then determined after 24 and 48 h of exposure at sublethal and lethal concentrations using 20 animals per concentration divided into groups of 10 shrimps per tank (experiment 2: dose effect after 24 and 48 h of exposure). In *P. vannamei*, the effect of sublethal and lethal concentrations of fenitrothion was determined after 24 and 48 h of exposure using 20 animals per concentration with 10 shrimps per tank (experiment 3: dose effect after 24 and 48 h of exposure). Juvenile *P. stylirostris* and *P. vannamei* used in these experiments weighed 13.5 ± 1.4 g and 14.2 ± 2.0 g, respectively.

Effect of Fenitrothion on Muscle Acetylcholinesterase Activity

In vitro acetylcholinesterase activities were determined in juveniles of *P. stylirostris* (13.5 ± 1.4 g) and *P. vannamei* (12.0 ± 1.1 g) after a 24-h period of exposure to fenitrothion at sublethal concentrations. Prior to enzyme activity determinations, samples (whole abdomens) were frozen for 48 h (exp. A) and 24 h (exp. B) at -80°C . Muscle tissues were suspended in 0.1 M Tris buffer, pH = 8 and homogenized for 20–30 sec using an Ultraturax homogenizer. Extracts were then centrifuged at 12,000 G for 10 min. Supernatants were immediately frozen at -20°C . Muscle homogenates were used to determine their protein content and AChE activity. AChE activity was determined according to the spectrophotometric method of Ellman et al. (1961) modified for microtitration plate reading (Automated microplate reader EL309, Bio-Tek Instruments) by Galgani and Bocquené (1988). For protein determination, the method of Bradford (1976) was employed using 0.1 M

Tris buffer, pH = 8 (Bocquené et al., 1990), and bovine serum albumin as standard. All essays were done in quadruplicate.

Statistical Analysis

Statistical comparisons of experimental data were performed by one-way and two-way analysis of variance (ANOVA) and Fisher's Least Significant Difference test (LSD.) by using the software Statview 4.01 Abacus Concept (ns: not significant; *p < .05, and **p < .01).

RESULTS

Fenitrothion Concentration in the Media

The results of titrations of fenitrothion concentrations performed on test solutions after 24 and 48 h of experiments are given in Table 1. Water samples were taken from parallel series without the animal. Fenitrothion concentrations varied moderately in the media with a decrease of 15–20% within 48 h. Nominal values of fenitrothion concentrations were used thereafter.

Acute Toxicity of Fenitrothion

LC₅₀ values of 24 and 48 h for the various larval, postlarval, and juvenile instars of both species are given in Table 2. The rate of nauplii mortality was below 50% in all tested fenitrothion concentrations, therefore their 24 and 48 h LC₅₀ values were over 500 µg fenitrothion.l⁻¹. Tolerance to fenitrothion then decreased in exposed larvae, from zoea to mysis instars. In *P. stylirostris*, 24 and 48 h LC₅₀ values ranged from 160 to 12 µg.l⁻¹ and from 42 to 2 µg.l⁻¹, respectively. In *P. vannamei*, 24 and 48 h LC₅₀ values ranged from 324 to 12 µg.l⁻¹ and from 29 to 10 µg.l⁻¹, respectively. The first postmetamorphic instar, PL1, of both species also showed high susceptibility to fenitrothion: 24 h LC₅₀ values were between 7.7 and 10 µg.l⁻¹. In juvenile instars, tolerance to fenitrothion increased with increasing wet weights. Juvenile *P. vannamei* showed higher fenitrothion tolerance (higher LC₅₀ values) than juvenile *P. stylirostris*; e.g., for *P. vannamei* and *P. stylirostris* juveniles of similar wet weight (14 g), 24 h LC₅₀ values were 28.5 µg.l⁻¹ and 17.6 µg.l⁻¹, respectively.

Effect of Fenitrothion on Osmoregulatory Capacity

In *P. stylirostris*

Experiment 1: short-term time effect. Hypo-OC of control juveniles remained stable over the 12 h experiment (Fig. 1). In juveniles exposed to 10 µg.l⁻¹, hypo-OC was not affected after 1 h but it decreased by 11, 17.3, and 22% compared with the controls at 3, 6, and 12 h of exposure, respectively. The effects of the 10

TABLE 1.

Fenitrothion loss (in %) in seawater according to initial concentrations and time. Number of samples per condition: 3.

Time (h)	Initial Concentrations (µg.l ⁻¹)			
	1 µg.l ⁻¹	2 µg.l ⁻¹	4 µg.l ⁻¹	20 µg.l ⁻¹
24	10	5	0	3
48	17	20	15	17

TABLE 2.

Penaeus stylirostris and *P. vannamei*: fenitrothion 24 and 48 hr LC₅₀ with 95% confidence limits for larval, postlarval, and juvenile instars.

		24 hr LC ₅₀ (µg.l ⁻¹)	48 hr LC ₅₀ (µg.l ⁻¹)
<i>P. stylirostris</i>			
Larval instars	N	>500	>500
	Z1	60 (39–97)	42 (30–62)
	Z2	160 (115–227)	24 (17–35)
	Z3	73 (52–104)	40 (25–71)
	M1	42 (30–61)	15 (10–24)
	M2	56 (40–83)	9 (6–13)
	M3	12 (7–17)	2 (1–3)
Postlarval instar	PL1	10 (6–15)	nc
Juveniles	8 g	11 (7–15)	11 (7–15)
	14 g	18 (13–25)	16 (11–26)
	20 g	19 (13–28)	nc
<i>P. vannamei</i>			
Larval instars	N	>500	>500
	Z1	324 (200–531)	23 (15–35)
	Z2	303 (175–676)	29 (20–43)
	Z3	282 (165–508)	12 (7–18)
	M1	169 (90–351)	28 (20–39)
	M2	20 (14–27)	15 (10–22)
	M3	12 (8–18)	10 (5–14)
Postlarval instar	PL1	9 (6–13)	8 (5–11)
Juveniles	7 g	19 (14–27)	19 (13–30)
	9 g	19 (10–33)	nc
	12 g	30 (20–46)	29 (20–42)
	14 g	29 (18–46)	nc
	18 g	42 (23–77)	nc

N: nauplii (III, IV, V), Z: Zoea, M: Mysis, PL1: postlarvae 1, nc: not calculated.

µg.l⁻¹ sublethal concentration on hypo-OC were significant within the 12 h of the experiment (F = 6.2, p = .0021). The Fisher Post-Hoc test shows that the decrease was significant 3 h after the introduction of fenitrothion into the test media.

Experiment 2: dose effect after 24 and 48 h of exposure. After 24 h, the hypo-OC of juveniles exposed to 6, 8, 10, 12, and 14 µg.l⁻¹ of fenitrothion decreased respectively by 7, 8, 10, and 22%

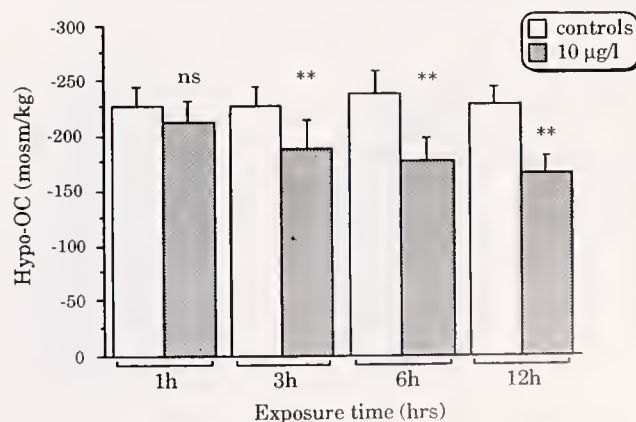


Figure 1. Effect of short-term exposure to 10 µg.l⁻¹ of fenitrothion for different periods (1 to 12 h) at 27°C in seawater (1060 ± 10 mosm.kg⁻¹) on hypo-osmoregulatory capacity (hypo-OC) of juvenile *Penaeus stylirostris* (13.5 ± 1.4 g) 7 < n < 12. The results are given as means ± SD.

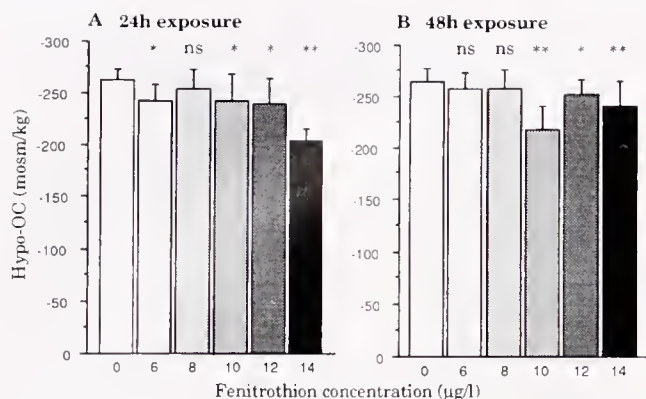


Figure 2. Effect of 24 h (A) and 48 h of exposure (B) to 0, 6, 8, 10, 12, and 14 $\mu\text{g l}^{-1}$ of fenitrothion at 27°C in seawater ($1060 \pm 10 \text{ mosm kg}^{-1}$) on hypo-OC of juvenile ($13.5 \pm 1.4 \text{ g}$) *Penaeus stylirostris*. $7 < n < 11$. The results are given as means \pm SD.

compared with the controls (Fig. 2A). After 48 h of exposure, the hypo-OC of juveniles exposed to 10, 12, and 14 $\mu\text{g l}^{-1}$ of fenitrothion also decreased by 18, 5, and 10%, respectively, compared with the controls (Fig. 2B). These hypo-OC decreases with increasing sublethal and lethal concentrations of fenitrothion were statistically significant for both times of exposure ($F = 9.19$, $p < .0001$ and $F = 7.89$, $p < .0001$, respectively).

In *P. vannamei*

Experiment 3: dose effect after 24 and 48 h of exposure. After 24 and 48 h of exposure, hypo-OC did not vary with increasing sublethal fenitrothion concentrations except at the lethal 25 $\mu\text{g l}^{-1}$ fenitrothion concentration where it decreased (Fig. 3A and 3B) ($F = 14.6$, $p < .0001$ after 24 h of exposure and $F = 3.29$, $p = .011$ after 48 h of exposure). The Fisher' post-Hoc test indicates that only the 25 $\mu\text{g l}^{-1}$ lethal fenitrothion concentration for both the 24 and 48 h experiments induced significant differences compared with the control group.

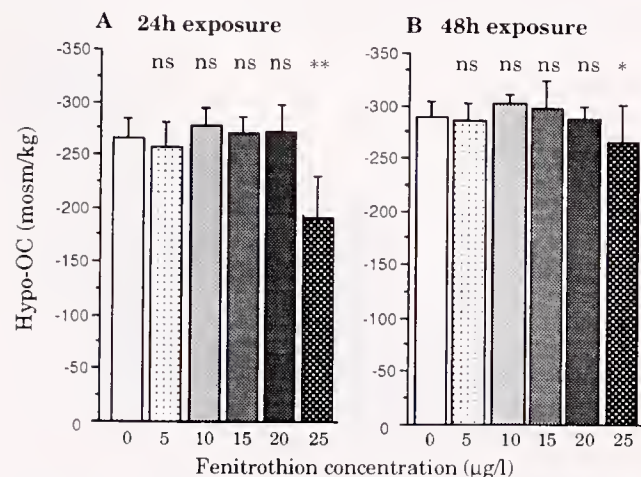


Figure 3. Effect of 24 h (A) and 48 h of exposure (B) to 0, 5, 10, 15, 20, and 25 $\mu\text{g l}^{-1}$ of fenitrothion at 27°C in seawater ($1060 \pm 10 \text{ mosm kg}^{-1}$) on hypo-OC of juvenile ($14.2 \pm 2.0 \text{ g}$) *Penaeus vannamei*. $7 < n < 15$. The results are given as means \pm SD.

Muscle Acetylcholinesterase Activity

In *P. stylirostris*

After 24 h of exposure, AChE activity decreased at all tested sublethal fenitrothion concentrations (4, 6, and 8 $\mu\text{g l}^{-1}$) by 18, 13, and 16%, respectively ($F = 3.032$; $p < .05$) (Fig. 4). AChE activity decreases were all significant compared to the controls.

In *P. vannamei*

After 24 h of exposure, AChE activity of shrimp showing no behavioral sign of contamination did not vary at sublethal and lethal fenitrothion concentrations ($F = 0.14$, $p = .93$) (Fig. 5A). In moribund shrimp, AChE activity sharply decreased with increased lethal fenitrothion concentrations ($F = 10.44$, $p = .0003$) by 15.5 and 30% at 20 and 30 $\mu\text{g l}^{-1}$, respectively (Fig. 5B). At these concentrations, AChE activities were significantly different compared with the controls. Differences in AChE activities were observed in controls between replicates A and B which had been subjected to different freezing periods (48 and 24 h) prior to enzymatic determinations.

DISCUSSION

Fenitrothion appeared very toxic to *P. vannamei* and especially to *P. stylirostris*. For both species, a rapid decrease in the fenitrothion tolerance was observed after 24 and 48 h of exposure from larval to juvenile instars. A similar high sensitivity towards fenitrothion has previously been reported for other crustacean species (Table 3). Among penaeid shrimps, fenitrothion appeared less toxic to *P. vannamei* and *P. stylirostris* than to *P. aztecus* and *P. japonicus* (McLeese, 1976; Kobayashi et al., 1985; Lignot et al., 1997). For instance, 24 and 48 h LC_{50} values ranged from 10.5 to 41.5 $\mu\text{g l}^{-1}$ for *P. stylirostris* and *P. vannamei* juveniles and from only 1.0 to 2.5 $\mu\text{g l}^{-1}$ for *P. aztecus* and *P. japonicus*.

The rapid decrease in fenitrothion tolerance observed in *P. stylirostris* and *P. vannamei* from the highly resistant nauplii to the Zoea 1 might originate from physiological changes. During the first 24 h after hatching, nauplii derive energy from yolk and thus

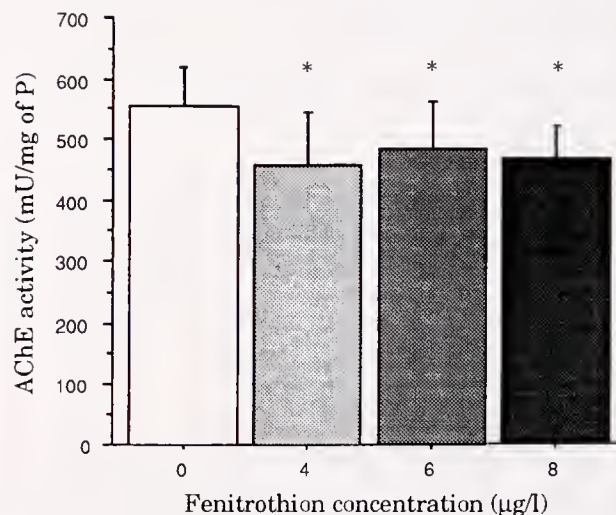


Figure 4. Effect of 24 h of exposure to 0, 4, 6, and 8 $\mu\text{g l}^{-1}$ of fenitrothion at 27°C in seawater ($1060 \pm 10 \text{ mosm kg}^{-1}$) on acetylcholinesterase activity (AChE) of juvenile ($13.5 \pm 1.4 \text{ g}$) *Penaeus stylirostris*. $n = 8$. The results are given as means \pm SD. AChE activity is expressed as mU/mg protein; 1 mU is 1 μmol of substrate hydrolyzed/l per min.

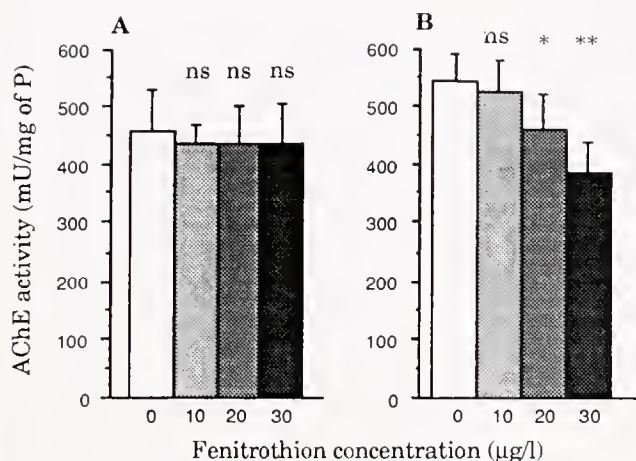


Figure 5. Effect of 24 h of exposure to 0, 10, 20, and 30 $\mu\text{g l}^{-1}$ of fenitrothion at 27°C in seawater ($1060 \pm 10 \text{ mosm kg}^{-1}$) on (AChE) activity of juvenile ($12.0 \pm 1.1 \text{ g}$) *Penaeus vannamei*. (A) Contaminated animals showing no sign of contamination; (B) moribund shrimps. $n = 6$. The results are given as means \pm SD. AChE activity is expressed as mU/mg protein; 1 mU is 1 μmol of substrate hydrolyzed/l per min.

probably experience minimal exchanges with the surrounding polluted medium. The increase in size when the nauplii molt to Zoea 1, involving a high uptake of water to extend the body volume, might explain the increase in fenitrothion toxicity. The toxicity is further enhanced with the progress of the postembryonic stages as observed in other decapods (McLeese, 1976; Kobayashi et al., 1985; Sarojini et al., 1986; Lignot et al., 1997). This increasing toxicity has been considered by Rompas et al. (1989) and Kobayashi et al. (1990) in *P. japonicus* as resulting from an increase in the oxydative disulfuration activity with the progress of larval instars: the growing shrimp would be able to transform fenitrothion in its oxon form (fenitrooxon), the latter being 10–20% more toxic than the original form. Inversely, the increased ability to tolerate the pesticide with the progress in size of juveniles might be related to other processes, such as those involved in insecticide-resistant insects. Enhanced detoxification, with metabolism of the toxic fenitrooxon to less toxic metabolites by nonspecific esterases and glutathione S-transferases, can possibly occur as in the mosquito *Anopheles subpictus* (Hemingway et al., 1991). Lower sensitivity to fenitrothion could also be attributed to an increased fenitrooxon hydrolysis as in the resistant caddisfly, *Cheumatopsyche brevilineata* (Konno and Shishido, 1994) and/or to a decreased effect on the acetylcholinesterase activity as in the resistant diamondback moth, *Plutella xylostella* (Konno and Shishido, 1996).

When juvenile *P. stylirostris* were exposed to lethal and sublethal concentrations of fenitrothion in seawater, their hypo-OC decreased significantly compared with the controls. Decreases in hypo-OC after sublethal fenitrothion exposure have previously been reported in *P. japonicus* (Lignot et al., 1997). Several studies have shown that the ability to osmoregulate was impaired in crustaceans exposed to adverse conditions or to different toxic compounds such as heavy metals (Thurberg et al., 1973; Jones, 1975; Bjerregaard and Vislie, 1985, 1986; Boitel and Truchot, 1989; Bambang et al., 1995a, Bambang et al., 1995b), hypoxia (Charmantier et al., 1994), low pH (Allan and Maguire, 1992), ammonia (Lin et al., 1991; Lin et al., 1993; Chen and Cheng, 1996). However, in a few species, osmoregulation was not affected by pollut-

ants. For instance, no impairment of the osmoregulatory status of *Myxidopsis bahia* was observed at sublethal cadmium concentrations in seawater and diluted seawater (DeLisle and Roberts, 1994). Similarly, the osmotic regulation in *Hemigrapsus nudus* and the osmotic and ionic regulation in *Cancer magister* were not impaired by sublethal acute exposures to the organochlorine methoxychlor (Caldwell, 1974).

A decrease in osmoregulatory capacity indicates an imbalance in the plasma ion concentrations. It is an indication of an inhibition of osmoregulatory mechanisms and/or of a failure of normal homeostatic processes. Metabolic alterations (e.g., inhibition of ATPases activity, change in the permeability of the gills, and epipodites to water and ions) could possibly be responsible for the hypo-OC alterations, as already described in crustaceans after exposure to organochlorine compounds (Reddy et al., 1992) and in fish species after exposure to organophosphorus insecticides and carbamates (Hiltibrant, 1982; Hohreiter et al., 1991). Among peneid shrimps, stresses such as turbidity and ammonia exposure are known, for example, to increase the shrimp $\text{Na}^+\text{-K}^+$ ATPase activity in gills and epipodites (Lin et al., 1992, 1993). However, no effect on the $\text{Na}^+\text{-K}^+$ ATPase activities of *P. japonicus* was recorded after lethal and sublethal exposure to tributyltin oxide (Lignot et al., 1998). The hypo-OC decrease observed in *P. stylirostris* might therefore be explained by histological changes (e.g., damage of epithelial cells) in osmoregulating tissues, as already observed in *P. japonicus* after exposure to sublethal fenitrothion concentrations (Lignot et al., 1997). The gill structure and ultrastructure were also impaired in *P. monodon* after exposure to lethal gusathion A concentrations (Baticados and Tendencia, 1991) and in *Carcinus maenas* after exposure to sublethal copper concentrations (Nonnotte et al., 1993; Lawson et al., 1995). The observed histological changes can increase the permeability of the gills and epipodites, resulting in an increased movement of water and ions.

The lack of impairment of the osmoregulation in *P. vannamei* after acute sublethal fenitrothion exposure, on the other hand, may indicate that osmoregulatory tissues such as gills and epipodites are not affected or that compensatory responses are involved. Adaptive changes in the metabolism of peneids such as enhanced gluconeogenesis and changes in protein metabolism might, for example, represent such compensatory mechanisms and may attenuate the toxic stress due to organophosphorus insecticides (Vijayalakshmi and Ramana Rao, 1985; Reddy et al., 1990).

The significant inhibitions of muscle AChE activity in *P. stylirostris* when juveniles were exposed to sublethal concentrations of fenitrothion correlate well with previous data obtained in crustaceans (Coppage and Matthews 1974; Reddy and Rao, 1988; Repetto et al., 1988; Morgan and Kiceniuk, 1990; Reddy et al., 1990; Bocquené and Galgani, 1991; Gälli et al., 1994). A lack of inhibition of AChE activities similar to the one observed in *P. vannamei* has previously been reported in *P. duorarum* exposed to lethal concentrations of methyl parathion (Schoor and Brausch, 1980) and in the stonefly, *Claassenia* sp., after acute exposure to fenitrothion at sublethal concentrations (Day and Scott, 1990). In the crayfish *Procambarus clarkii*, the muscle AChE activity even slowly increased within the first 3 days of exposure to a sublethal concentration of trichlorfon and then abruptly decreased after 4 days of exposure (Repetto and Repetto, 1988). In *P. vannamei*, the observed differences for the AChE activities in the controls of the two replicates can be due to the different freezing periods (24 and 48 h) of the samples prior to the enzymatic determinations. Freezing of the whole animal may significantly alter AChE activity as

TABLE 3.
Lethal concentrations (LC₅₀) of fenitrothion in different crustacean species.

Species (stage)	Exposure (h)	Salinity (PSU)	T (°C)	LC ₅₀ (µg l ⁻¹)	Reference
<i>Daphnia pulex</i>	24	FW	20	45	McLeese (1976)
<i>Gammarus lacustris</i>	24	FW	20	60	Symons (1976)
<i>Penaeus aztecus</i>	48	SW	?	2.5	McLeese (1976)
<i>Penaeus japonicus</i>					
Juveniles	24	18.3	24–25	1	Kobayashi et al. (1985)
Larvae (zoae 1-mysis 3)	24	37	28	17–11	Lignot et al. (1997a)
(zoea 3-mysis 3)	48	37	28	4–2	
Post-larvae (PL1-PL15)	48	37	28	1.8–0.5	
(PL3-PL15)	96	37	28	0.1–0.5	
Juveniles (11 g)	24	36	25	1.9	
	48	36	25	1.5	
	96	36	25	0.8	
<i>Palaemonetes paucidens</i>	96	FW	25 ± 2	2.2	Takimoto et al. (1987)
<i>Palaemonetes varians</i>	48	SW	?	6	Symons (1976)
<i>Crangon crangon</i>	48	SW	?	3	Symons (1976)
<i>Paryata compressa improvisa</i>	48	FW	22 ± 1	1.2	Hatakayama and Sugaya (1991)
<i>Macrobrachium kistmensis</i>	96	FW	28 ± 2	0.97	Pawar and Katdare (1982)
	96	FW	23 ± 1	0.91	Pawar and Katdare (1984)
<i>Macrobrachium lamerii</i>					
Larvae	24	FW	?	4.14 ^a	Sarojini et al. (1986)
	48	FW	?	4.22 ^a	
	96	FW	?	4.42 ^a	
Juveniles	96	FW	29 ± 1	1.085	AvelinMary et al. (1986)
Adults (male)	96	FW	29 ± 1	0.545	
Adults (female)	96	FW	29 ± 1	0.631	
<i>Homarus americanus</i>					
Larvae	24	SW	15	100	McLeese (1976)
Adults	24	SW	15	10–100	
	96	SW	15	1	
<i>Orconectes limosus</i>					
Small (32 mm total length)	24	FW	12	32	McLeese (1976)
	96	FW	12	10	
Large (63 mm total length)	24	FW	12	>100	
	96	FW	12	30	
<i>Carcinus maenas</i>	48	SW	?	10	Symons (1976)
<i>Callinectes sapidus</i>	96	34	22	8.6	Johnston and Corbett (1985)
<i>Oziotelphusa senex senex</i>	48	FW	25 ± 1	400	Bhagyalakshmi and Ramamurthi (1980)

FW: freshwater; SW: seawater (when no PSU values are given by the authors).

^a LC₅₀ values for *Macrobrachium lamerii* (Sarojini et al., 1986) are estimated from graphs.

previously observed (Finlayson and Rudnicki, 1985). However, only a relative error occurs provided that control and treatment groups are handled in the same manner.

The different AChE sensitivity between *P. stylirostris* and *P. vannamei* can be attributed to different affinity and phosphorylation rates of AChE. Fenitrothion may also be more rapidly hydrolyzed and/or excreted in *P. vannamei* than in *P. stylirostris*. This species-specific difference in AChE sensitivity may therefore be explained in relation to the AChE affinity and to different biotransformation rates of the fenitrothion, as in fish species (Keizer et al., 1995).

In conclusion, fenitrothion has a selective toxicity among penaeid shrimps. *P. stylirostris* appeared more sensitive to fenitrothion than *P. vannamei*. In *P. stylirostris*, osmoregulation and AChE activity were impaired after sublethal acute exposure. In *P. vannamei*, sublethal exposure to fenitrothion did not alter the osmoregulatory capacity and the muscle AChE activity. In light of

these results, osmoregulatory capacity and muscle AChE activity—that have been considered valuable tools to monitor the physiological state of crustacean species under sublethal conditions due to an organophosphorus insecticide exposure (Lignot et al., 1997)—can also be used in *P. stylirostris* but not in *P. vannamei*. The observed species-specific toxicity of fenitrothion might have a multifactorial dependence. Further work must therefore be carried out in order to gain a better knowledge of the possible metabolic routes leading to the observed selective toxicity of fenitrothion among penaeid shrimps.

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A DEVICE FOR ACCURATE AND RAPID SIZE MEASUREMENTS OF MOLLUSCS

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ABSTRACT The construction and use of a measuring device for hard-shelled molluscs is described. The instrument is easy to use and has an accuracy of ± 0.1 mm. Its advantage over a caliper ruler or other commonly used devices (i.e., measuring boards) is that it allows for measurement of large numbers of specimens in a very short time without losing accuracy in the individual measurement.

KEY WORDS: Measuring box, hard shelled molluscs, integrated caliper rule, size measurements

INTRODUCTION

Population studies require large number of individual size measurements to obtain representative and statistically sound data (Pauly, 1985; Sparre and Venema, 1987). In most instances, time and manpower is limited, however. The consequence is often that either the number of individual (accurate) measurements taken is too small or that many measurements are taken at the expense of accuracy.

Fisheries scientists and aquaculturists studying the dynamics of mollusc populations know this problem from their field work on vessels, at landing places, at the fish market, or at aquaculture farms. In addition, fisherman and resellers usually do not like to spend hours watching scientists measure their precious shellfish.

The caliper rule seems to be the most accurate measuring device, but the handling can be time consuming, especially when dealing with animals of irregular shape. Other methods such as measuring boards or boxes allow for faster measurements but they are less accurate. In order to overcome this problem we designed a measuring box with an integrated caliper rule which allows for rapid and easy measurement of hard-shelled organisms (Plate 1). Its accuracy is ± 0.1 mm, the design is simple, and it is easy to clean. With this device up to 600 snails (*Thais coronata coronata*) can be measured in about 1 h. The original version of this device was a box with a scale and an included slide, which was used by one of us (M. Wolff) to measure commercially harvested *Argopecten purpuratus* in Perú back in the early 80s (Wolff, 1985). It provided fast handling but low accuracy (± 1 mm), so we decided to change the design.

CONSTRUCTION OF THE DEVICE

The Box

According to the measurements in the diagram (Fig. 1a, b) the box was cut of a solid PVC-block with a CNC milling machine. We used PVC as it is relatively cheap, easy to work, and resistant to seawater. Another advantage is that the finished device weighed little more than 1 kg. At the side where the caliper rule is attached, 3 mm of the PVC-block had to be cut out to provide space for the moving part of the caliper rule (Fig. 1a). Stainless steel screws (M6, 20 mm length) were used for attaching the caliper rule to the box. The attachment was fixed with M5 screws (20 mm) to the

movable part of the caliper rule. The holes were drilled with a helicoil drill and helicoil threads were then inserted. These metal threads keep the pitches from wearing out when the caliper rule has to be dismantled for cleaning. Drainage holes (5 mm) were drilled into the bottom of the box. The attachment (Fig. 1d) was also made of PVC, with metal threads inserted into the drill holes, as described above. Rubber patches were glued to the bottom of the box to provide hold on smooth surfaces.

The Caliper Rule

A sturdy caliper rule (stainless steel) of about 300 mm in length was used. A good quality is recommended as it is crucial for the overall stability of the device. After being cut to the desired length, the holes were eroded into the tempered steel, as indicated in Figure 1c. We strongly recommend that the device be professionally made as its accuracy depends on exact construction. A precision engineer can build the device on the basis of the technical drawing (Fig. 1).

USING THE DEVICE

The size of our tool was chosen for animals between 10.0 and 100.0 mm. The main advantage is that the caliper rule is fixed and the attachment provides a large area for measuring animals. Furthermore, the box can be used to position animals correctly, which is sometimes difficult when using a normal caliper rule. Measuring animals has to be done with care, as the attachment is fixed only with two screws and is the most sensitive part of the whole device. Its durability depends largely on how carefully it is used. We had no problems during a 1 ½ year study in northern Brazil where several thousand animals were measured. To keep the metal parts from corroding, a good oil (e.g., Ballistol) should be applied before and after each use. This guarantees smooth action of the movable parts and protects the metal from the seawater.

We are confident that the interested reader will find this instrument very useful.

ACKNOWLEDGMENTS

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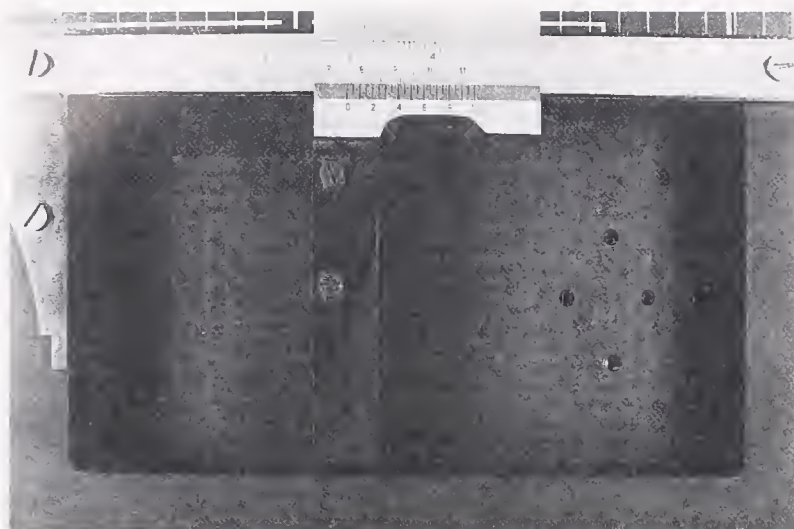


Plate 1. Shows the top view of the finished (and well used) device.

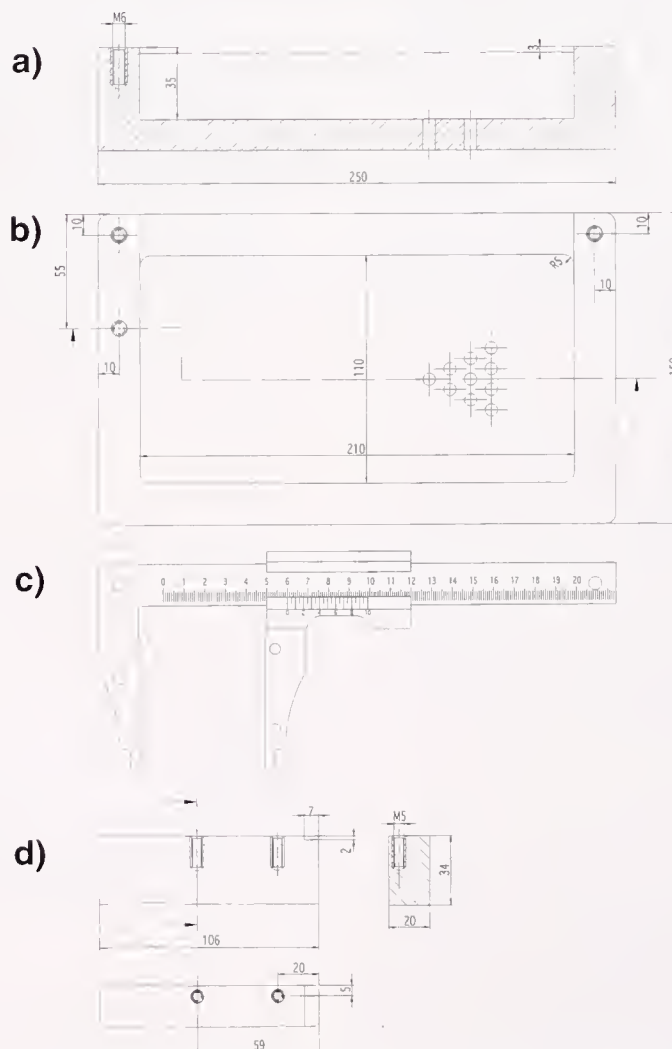


Figure 1. Technical drawing of the device (a) from the side where the caliper rule is attached, (b) from above, (c) drawing of the caliper rule, and (d) of the attachment. All measurements are given in mm.

ernmental Agreement on Cooperation in the Field of Scientific Research and Technological Development between Germany and Brazil. It was financed by the Germany Ministry for Edu-

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ABSTRACTS OF TECHNICAL PAPERS

Presented at the 51st Annual Meeting

PACIFIC COAST OYSTER GROWERS ASSOCIATION

NATIONAL SHELLFISHERIES ASSOCIATION

(Pacific Coast Section)

Newport, Oregon

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THE EFFECTS OF MOWING *SPARTINA ANGLICA* DURING NOVEMBER, DECEMBER, JANUARY AND FEBRUARY IN TRIANGLE COVE, CAMANO ISLAND, WASHINGTON. Jerry Bentler, Tulalip Tribes Fisheries Department, 7615 Totem Beach Road, Marysville, Washington 98271.

Preliminary tests by Bentler (1997) indicated potential for high efficacy using winter mowing as a control methodology for *Spartina anglica* and *S. alterniflora* in North Puget Sound. Four test plots in Triangle Cove, Camano Island, Washington, were cut during November and December of 1996, and January and February of 1997 to test the feasibility of use for larger, production scale control. Each plot received two treatments and was compared to a control. The first treatment was an initial cutting with a sickle bar mower. The second treatment occurred after flushing by the tide and consisted of mowing to the substrate with weed eaters.

Stem counts of randomly selected plots were conducted in July, 1997. Data indicates that November and December treatments had the greatest efficacy. January had almost no effect, and February treatments may stimulate growth. Further cutting experiments are recommended for November, and perhaps October.

RESULTS OF VOLUNTEER BEACH ASSESSMENT PROGRAM ON THE PUGET SOUND BEACHES OF KING COUNTY, WASHINGTON. Robert N. Brenner, King County Department of Natural Resources.

Citizen volunteers were recruited via news releases to local newspapers and radio stations to collect various data which were used to assess the current condition of the local beaches and to determine whether significant changes had occurred over the twenty years since two investigators surveyed five of the beaches as part of their doctoral research/thesis work. The volunteers assisted with or accomplished invertebrate surveys and seaweed surveys. The volunteers also conducted clam population estimates following the Washington Department of Fish and Wildlife sampling protocol. The volunteers collected data used to draw topographic maps of most of those beaches. Most of the seventeen beaches in the study area have been surveyed twice over the past three years.

The data did not show significant changes in the invertebrate populations or the sea weeds. There was evidence of destruction of the beaches and declines in the numbers and sizes of various invertebrate and algae target species which are attributable to over-enthusiastic harvesting and poaching.

It was found that using volunteers is a cost effective avenue for conducting labor intensive surveys. This is also a very effective educational tool.

QUILCENE-BRINNON SCHOOLS SHELLFISH SCIENCE CLUB: A MODEL SHELLFISH FARM ON HOOD CANAL.

Colleen A. Burge and Amalia C. Baker, Seniors at Quilcene High School, Quilcene, WA 98376. John Pitts and Charles Baker, Advisors

The Quilcene-Brinnon Schools Shellfish Science Club is an educational, high school club that raises oysters and teaches the community about water quality. The oysters are raised on both Quilcene and Dabob bays off the Hood Canal. 4.7 acres of beach was donated by Jefferson County and the Washington Department of Fisheries and Wildlife. The oysters raised are sold at fairs, festivals and an annual community dinner. At these functions water quality brochures are handed out to stress the importance of clean water to the community.

Student members have a variety of educational activities for participation, from business procedures to labor intensive work. The club's main goal is to raise enough money to take a fun and educational, aquaculture related field trip every two years.

Quilcene High School students have managed and operated the model farm for over three years, receiving two environmental awards, one locally and one nationally. The club hopes to continue to raise oysters and teach the community about the importance of water quality.

WESTERN REGIONAL AQUACULTURE INDUSTRY SITUATION AND OUTLOOK REPORT: A SHELLFISH PERSPECTIVE. Kenneth K. Chew, William K. Hershberger, and Derrick R. Toba, Western Regional Aquaculture Center, School of Fisheries Box 357980, University of Washington, Seattle, WA 98195-7980.

A survey to estimate the aquaculture production in the twelve western states was conducted for the Western Regional Aquaculture Center (WRAC). The western region includes Alaska, Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington and Wyoming. This study was designed to 1) provide production estimates for all species cultured, and identify the states in which they are produced; 2) provide information on the current status of each industry and estimate future (year 2000) production; and 3) determine product forms sold by the producers and provide the average selling price. Information on finfish (salmon, trout, catfish, tilapia and others), shellfish (oysters, mussels and others), aquatic plants and non-foodfish were collected for the survey.

Total aquaculture production for the western region was 153,000 pounds, which was valued at \$181,000. The three leading states, Washington (72 million pounds), Idaho (45 million pounds) and California (27 million pounds) combined to produce over 94% of the total weight. California (\$56 million), Idaho (\$57 million) and Washington (\$56 million) combined to account for 94% of the total value of aquaculture production in the western region.

Of the total western region aquaculture production, 67 million

pounds (44%) and valued at \$20 million (11%) were shellfish. Five states reported commercial shellfish aquaculture harvests and sales: Alaska, Arizona, California, Oregon, and Washington. In each of the coastal states, oysters comprised the majority of shellfish production. Other species harvested included mussels, clams, scallops, abalone, crawfish, and shrimp. Trends over the past ten years were analyzed for each state and species.

A STOCK REBUILDING PLAN FOR THE NATIVE OLYMPIA OYSTER (*OSTREA LURIDA*) ON PUBLIC TIDELANDS IN WASHINGTON STATE. Anita E. Cook and J. Anne Shaffer, Washington State Department of Fish and Wildlife, 1000 Pt. Whitney Road, Brinnon, WA 98320; Brett R. Dumbauld and Bruce E. Kauffman, Washington State Department of Fish and Wildlife, P.O. Box 190, Ocean Park, WA 98640.

The Olympia oyster (*Ostrea lurida*) is native to the state of Washington. Its numbers, as well as distribution were drastically reduced after European settlement, predominately due to overharvest and pollution. The non-native Pacific oyster (*Crassostrea gigas*), which is relatively easily cultured and reproduces naturally in the Hood Canal area and Willapa Bay, has become the most abundant oyster in Washington state. Pacific oysters represent over 99% of the commercial oyster harvest.

The Washington State Department of Fish and Wildlife (WDFW) is currently investigating the status of the Olympia oyster, with the goal of increasing native oyster populations on public tidelands where they once existed and assuring that they do not become threatened or endangered. WDFW is developing an Olympia oyster stock rebuilding plan. We give a brief synopsis of the plan to date including historical background and current distribution of the native oyster, management, harvest, predators, genetic considerations, and habitat and water quality issues. Finally, we summarize the goals and objectives of the rebuilding plan and some proposed actions in hopes of stimulating discussion among representatives from the oyster industry, academia, tribes, government agencies and other affected parties.

ROCK SCALLOP CULTURE: THE IMPORTANCE OF ATTACHMENT. Carolyn S. Culver, John B. Richards, and Henry M. Page, Department of Ecology, Evolution and Marine Biology and Marine Science Institute, University of California, Santa Barbara, California 93106.

Many are excited about the potential culture of the rock scallop, *Crassadoma gigantea* (formerly *Himmites multirugosus*). With recent advances in larval rearing techniques, seed will soon be available for experimental grow-out. However, knowledge of effective grow-out methods are still lacking. Unlike other scallops, *C. gigantea* typically cements to a hard substrate as a juvenile (at a shell length of approximately ≥ 20 mm). The biological significance of

this cementing stage is little understood. Furthermore, factors regulating the cementing process are unknown. Understanding the biological importance of cementation and determining whether the process can be manipulated will be required before efficient grow-out methods can be developed.

Initial studies have examined the importance of attachment and scallop orientation at various current speeds. At high current speeds (≥ 9 cm/sec) scallops preferred to be vertically oriented within the water column. Preference for a specific orientation in relation to water flow, presumably one which optimizes feeding, was not as strong as originally suspected. In addition, scallop orientation to water flow did not change in response to a change in flow direction. The results and implications of these studies, which required the construction of specially designed experimental flow tanks, will be discussed. In addition, potential factors regulating the cementing process will be described. Our observations on attachment have prompted us to believe that manipulation of the cementing stage will be required for development of effective grow-out methods.

COMPARISON OF TWO PSP TOXICITY TESTING METHODS, ANATOMICAL DISTRIBUTION, AND INDIVIDUAL VARIABILITY IN PSP TOXINS IN THE GEODUCK CLAM. Kelly M. Curtis, University of Washington, School of Fisheries, Box 357980, Seattle, WA 98195.

The geoduck clam, *Panope abrupta*, is a valuable economic resource in the state of Washington where it is harvested and sold as a food product. However, the clam bioaccumulates the PSP producing dinoflagellate, *Alexandrium catenella*, which increases the risk of PSP poisoning to consumers, and may reduce its value as a food item for human consumption. Historically, levels of PSP toxin in the tissues of geoducks were not considered a problem; it was assumed that the gut was thrown out. In addition, traditional 'hot' areas of Puget Sound were closed to harvesting. Now, it has been learned that various Asian and Tribal communities are actually consuming the gut, and the traditional 'hot' areas where *A. catenella* blooms occur are being opened to harvest.

From August through December, 1997, geoducks will be collected from two 'hot' tracts within Puget sound. Neck, mantle and visceral ball will be tested separately for toxicity, using the receptor-binding assay and the mouse bioassay. Analyses of the data will address these questions, and will be discussed: What is the anatomical distribution of PSP toxins in geoduck? How do the receptor-binding assay and the mouse bioassay compare in testing for PSP? What is the clam to clam variability, does it vary with depth or season, and is it consistent between the two tracts?

New information regarding variability in geoducks may lead to a revision of the current monitoring program for PSP in geoducks, protect public health, minimize product recall, and minimize loss of income to state and tribal harvesters.

BURROWING SHRIMP CONTROL AND EELGRASS DISTRIBUTION IN WASHINGTON STATE COASTAL ESTUARIES. Brett R. Dumbauld, Washington State Department of Fish and Wildlife, P.O. Box 190, Ocean Park, WA 98640, Sandy Wyllie-Echeverria, School of Marine Affairs, University of Washington, Seattle, WA 98195.

The distribution of eelgrass (*Zostera spp.*) in coastal estuaries of Washington state is directly influenced by oyster aquaculture practices. Though little researched, harvest practices such as dredging are suspected to cause direct loss. In contrast, we observed enhancement in areas where the pesticide carbaryl had been applied to control burrowing thalassinid shrimp (*Neotrypaea californiensis* and *Upogebia pugettensis*) and began an experimental investigation into the mechanism of shrimp/eelgrass interaction.

Results from small scale intertidal spray experiments conducted in an area where *Neotrypaea* is very abundant suggest that eelgrass seeds sprout equally well in areas where shrimp are present and where they have been controlled with carbaryl. Subsequent growth of small sprouts however, is inhibited by the presence of shrimp and the young plants ultimately perish as shrimp become active in the spring. Oyster harvest activity typically occurs in the fall when eelgrass has begun to senesce and disperse seed. All of these considerations must be taken into account when managers weigh the functional results of aquaculture practices at the larger estuarine ecosystem scale.

THE NASCENT INVASION OF GREEN CRAB (*CARCINUS MAENAS*) IN WASHINGTON STATE COASTAL ESTUARIES. Brett R. Dumbauld and Bruce E. Kauffman, Washington State Department of Fish and Wildlife, P.O. Box 190, Ocean Park, WA 98640.

The presence of green shore crab (*Carcinus maenas*) which are native to Europe was documented in coastal estuaries of Washington state, U.S.A. during the summer of 1998. All crab appear to be of approximately the same age class having presumably recruited to the estuaries in 1997. Their present distribution with abundance greatest near the mouth of these estuaries appears to implicate larval transport along the Pacific coast as the probable vector for introduction.

Surveys conducted during summer 1998 suggest that the present distribution is restricted to the low intertidal salt marsh where potential competitors, particularly larger Dungeness crab are less abundant. Green crab were most abundant in *Spartina*, another invasive species which has clearly altered the intertidal environment in Willapa Bay. No green crab were found in subtidal channels or in areas where oyster aquaculture was being practiced, but this may be due to the early level of infestation (highest average = 2 crab/trap/24 hr). A plan for continued monitoring and potential control of green crab in Washington estuaries is outlined.

USE OF OYSTER SHELL TO CREATE HABITAT FOR JUVENILE DUNGENESS CRAB IN WASHINGTON COASTAL ESTUARIES: STATUS AND PROSPECTS. B. R. Dumbauld and R. E. Kauffman, Washington Department of Fish and Wildlife, Willapa Bay Field Station, P.O. Box 190, Ocean Park, WA 98640, D. A. Armstrong and E. Visser, School of Fisheries, Box 357980, University of Washington, Seattle, WA 98195, L. Cole-Warner, Seattle District U.S. Army Corps of Engineers, P.O. Box 3755, Seattle, WA 98124.

The deployment of oyster shell in estuarine intertidal areas to create habitat for juvenile Dungeness crab (*Cancer magister*) is now routinely used as a mitigation technique for "unavoidable losses" of crab during dredging operations in Grays Harbor and Willapa Bay along the southwest coast of Washington State. Original feasibility studies were conducted in 1986/87 for a project administered by the U.S. Army Corps of Engineers which widened and deepened the navigation channel in Grays Harbor. Since that time, several studies have elucidated the ecology of crab and other organisms that recruit to the shell reefs that are created and have also refined the procedures used to calculate the number of crabs lost due to dredging and those produced by the shell placement. While the shell produces crab habitat, initial assumptions about the longevity of the shell have proved to be overly optimistic and the shell often sinks or is covered with silt before the end of the first summer after deployment. In addition, competition with shore crabs *Hemigrapsus oregonensis*, has caused juvenile Dungeness crab to be displaced. We summarize results of these studies, make some ecological comparisons of habitat value with "natural" habitats such as eelgrass that also act as intertidal crab nursery areas, and present initial results from an ongoing mitigation effort which seeks to produce a more persistent living oyster reef in Willapa Bay.

POLYCULTURE OF *HALIOTIS RUFESCENS* AND *PALMARIA MOLLIS* UNDER REDUCED FLOW CONDITIONS AND ARTIFICIAL ILLUMINATION. Ford Evans and Chris J. Langdon, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Seasonal rates of dulse (*Palmaria mollis*) consumption by 10 to 80 mm red abalone (*Haliotis rufescens*) were compared with dulse growth rates under three flow conditions (1, 6, and 35 water volume exchanges per day) and three light regimes (natural light alone, natural light supplemented with either 12 or 24 h artificial illumination). These consumption and production measurements were used to estimate maximum possible stocking densities of red abalone cultured with dulse as a food source and in situ biofilter.

Dulse production declined with reduced flow rate during spring and summer, but not during winter. The use of supplemental illumination had a significant ($p < 0.05$) beneficial effect on dulse production, especially in winter when dulse production ranged from 14 g wet wt. $m^{-2} d^{-1}$ with natural light alone to 320 g wet wt.

$\text{m}^{-2} \text{d}^{-1}$ with 24 h supplemental illumination. The rate of dulse consumption by abalone can be expressed as a function of abalone whole body wet weight regardless of season (winter, spring and summer).

A maximum of 4,300 10 mm abalone could be sustained per 110 l culture container (stocked at 9 g l^{-1} dulse) during spring under 24 h artificial illumination and 35 water volume exchanges d^{-1} . Only 80 10 mm abalone could be sustained per culture container during winter under ambient light conditions and one water volume exchange d^{-1} .

BURROWING SHRIMP RECRUITMENT TO ESTUARINE INTERTIDAL HABITATS. Kristine L. Feldman and David A. Armstrong, School of Fisheries, Box 357980, University of Washington, Seattle, Washington 98195, Brett R. Dumbauld, Washington State Department of Fish and Wildlife, Willapa Bay Lab, P.O. Box 190, Ocean Park, Washington 98640.

Ghost shrimp, *Neotrypaea californiensis*, and mud shrimp, *Upogebia pugettensis*, are common in estuarine sediments along the Pacific coast of North America. Although both species infaunal burrowers, they differ in a number of life history characteristics such as timing of postlarval settlement, feeding mode, and burrow morphology, which may differentially affect their patterns of recruitment to intertidal habitats. This paper will review on-going studies investigating different aspects of burrowing shrimp recruitment to Washington coastal estuaries such as spatial and temporal patterns of distribution, recruitment to intertidal shell and mud substrates, and effects of oyster culture practices on abundance of young-of-the-year shrimp.

INTRASPECIFIC COMPETITION WITHIN SCALLOP SPAT COLLECTOR BAGS. Marcel Fr  chette, Institut Maurice-Lamontagne, Minist  re de P  ches et des Oc  ans, Mont-Joli, Qu  bec, Canada, G5H 3Z4

Scallop culture usually involves three steps: spat collection, intermediate culture in pearl nets and finally growout or bottom seeding. Using pearl nets is costly. The scallop industry in Magdalen Islands, Qu  bec, has been considering to perform intermediate culture in the spat collector bags, thus bypassing the pearl nets phase. To be efficient, this must be done at population densities high enough to be profitable, while minimizing intraspecific competition. Testing for intraspecific competition in controlled experiments poses insurmountable practical difficulties because of uncertainty in spatfall intensity. Therefore the intensity of competition was assessed from the relationship between body size and population density. Installing collector bags at different depths in the water column provided initial density groups spanning one order of magnitude. Preliminary analysis shows that competition among scallop spat did occur, but not at all depths in the water column.

IDENTIFYING THE KUMAMOTO OYSTER: A FIRST STEP IN BROODSTOCK RESTORATION AND IMPROVEMENT. Patrick M. Gaffney, University of Delaware, Lewes, DE 19958, Dennis Hedgecock, University of California Bodega Marine Laboratory, Bodega Bay, CA 94923, Anja Robinson, Hatfield Marine Science Center, Newport, OR 97365.

The Kumamoto oyster, *Crassostrea sikamea*, is a valuable part of the oyster industry in the Pacific Northwest, where it was introduced inadvertently with shipments of seed oysters (*C. gigas*) from Japan earlier this century. Although a number of growers have broodstock Kumamoto oysters, previous work has raised concern that their genetic integrity may be threatened by two factors: hybridization with the Pacific oyster *C. gigas*, and loss of genetic variability as a result of small effective population sizes.

As the first step towards preserving and improving the existing Kumamoto broodstock population, growers want to identify non-destructively oysters that are pure *C. sikamea* and separate them from *C. gigas* or hybrid oysters. Analysis of DNA markers amplified by the polymerase chain reaction (PCR) is an attractive alternative method that can be applied to small tissue snips, eggs and larvae. Previously, Banks et al. (1993) demonstrated the use of PCR-amplified mitochondrial genes to distinguish between Pacific and Kumamoto oysters. However, this approach cannot be used to identify hybrids, which possess only the maternal mtDNA genotype. For this reason, we developed a nuclear gene marker that can discriminate between the two species and their F1 hybrids.

We have typed several hundred biopsied oysters supplied by growers. Some lots contained substantial numbers of Pacific oysters, while others were almost pure Kumamoto. Although the relationships among surviving Kumamoto populations are not yet known, the prospects for a broodstock restoration program are good. To date, no hybrids have been found. This suggests either that hybridization was never that extensive, that growers have learned to be more discriminating, or possibly that hybrids do not survive well in the field.

THE GROWTH AND RECRUITMENT RATES OF BUTTER CLAMS ON SELECTED BEACHES IN THE STATE OF WASHINGTON. Stuart A. Goong, School of Fisheries, University of Washington, Seattle, WA 98195.

The butter clam (*Saxidomus giganteus* Deshayes) is a highly valued recreational shellfish species, but the commercial harvest has been historically small in Washington. However, harvests have increased in recent years, and basic information on the ecology and biology of the clam is necessary to establish a sound management regime. The primary objective of this study will be to determine the growth and recruitment rates of butter clams on three beaches in the Puget Sound Basin. The beaches that are included are Birch Bay State Park, Double Bluffs Beach, and Potlatch State Park. The beaches represent the northern, middle and southern portions of the

Puget Sound Basin, respectively. The growth rates will be determined by measuring lengths-at-age for clams collected from each beach. The recruitment rates will be determined by examining the age distributions of the clams from each beach. Examination of external shell annuli is central to the investigation. The annuli represent annual checks in the shell which can be used to age the clams. They will therefore allow determinations of both lengths-at-age and age distributions. A small number of shells will be thin sectioned in order to allow finer resolution in annuli. Annuli from these thin sections will be compared to external annuli from the same clams in order to verify that they represent annual checks. Secondary objectives in this investigation include the determination of clam size distributions for the beaches in this study, the examination of the hinge ligament for annual checks and the examination of any relationship between shell thickness and age. After a clam is aged fifteen to twenty years, the resolution in new annuli becomes very poor. Hinge ligament checks or increased shell thickness may allow a more refined understanding of the age distribution of this clam. The growth and recruitment rates of butter clams in Washington are poorly understood. This research will increase our understanding of butter clam biology and therefore contribute to a scientifically sound management strategy.

SURVIVAL, GROWTH, AND PATTERNS OF SEXUAL MATURATION OF TASMANIAN PACIFIC OYSTERS, *CRASSOSTREA GIGAS*, IN WASHINGTON STATE. Manfred T. Kittel and K. K. Chew, School of Fisheries, Box 357980, University of Washington, Seattle, WA 98195.

The F_1 generation of Tasmanian Pacific broodstock oysters, *Crassostrea gigas*, transferred from Tasmania to Washington state in 1994, was studied in comparative growout trials and laboratory analyses. Survival, growth, and shell morphology data were collected over an 18-month period from the Tasmanian oysters and control *C. gigas* of local origin. Seasonal changes in gonadal proliferation and glycogen storage were determined by quantitative histological examination and biochemical analysis of glycogen content. Results of these studies show that the F_1 progeny of the introduced oysters experienced significantly fewer mortalities at one of the experimental growout sites. In addition, the Tasmanian oysters attained a significantly greater shell length, whole volume, and whole weight than the controls. Patterns of gonadogenesis and glycogen storage are presented. A molecular genetic analysis by allozyme electrophoresis and PCR-RFLP methodology to characterize the introduced oysters as a distinct population is in progress. Based on their performance during the experimental growout, these Tasmanian oysters may be of value to oyster growers as distinct stocks or as broodstock for intraspecific hybridization.

SURVIVAL OF OYSTER (*CRASSOSTREA GIGAS*) LARVAE EXPOSED TO RODEO AND THE SURFACTANTS LI700, R11, AND X77. K. M. Kubena and C. E. Grue, Washington Cooperative Fish and Wildlife Research Unit, University of Washington, School of Fisheries, Box 357980, Seattle, WA. T. H. DeWitt, Battelle Pacific Northwest Laboratory, 1529 West Sequim Bay Road, Sequim, WA.

Smooth cordgrass (*Spartina alterniflora*), native to the Atlantic Coast, was accidentally introduced into Willapa Bay, in the late 1800s. Its distribution in the Bay has recently increased dramatically displacing mudflat habitat critical to a variety of fish and wildlife, including the commercially important Pacific oyster. Efforts to control *Spartina* have been complicated by concerns over potential non-target effects of Rodeo, the only herbicide approved for use on *Spartina* in Washington. Both glyphosate, the active ingredient of Rodeo, and the commonly used surfactants are known to adsorb to sediments. We conducted sediment toxicity tests with Pacific oyster pediveliger larvae in which larvae were exposed for 4 days to sediments spiked with Rodeo and the surfactants. The median lethal concentrations of the tank mixes were between 3,400 and 5,000 ppm glyphosate and varied with the surfactant added. Survival of larvae exposed to 3,353 ppm glyphosate and X77 was statistically different from controls, whereas tank mixes with LI700 and R11 were less toxic (statistically different from controls at 5,030 ppm). Toxic levels identified in the present study are more than two orders of magnitude less than maximum levels (16 ppm dry weight) of glyphosate detected in sediment after hand and aerial applications to control *Spartina* in Willapa Bay.

UPDATE ON THE MOLLUSCAN BROODSTOCK PROGRAM. Chris J. Langdon, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

The primary focus of the Molluscan Broodstock Program (MBP) is the genetic improvement of Pacific oysters through genetic selection. Pairs of oysters from either Willapa or Dabob Bay "wild" populations were crossed to produce about 150 full-sib families. The families were planted at five commercial sites along the West coast, U.S.A. Survival, growth and meat yields of planted families will be compared when they reach market size and top performing families will be used to produce the next MBP generation.

Interim live weights of families planted in 1996 were measured at sites in south Puget Sound and Willapa Bay in summer 1997 to determine if it will be possible to predict final rankings of families at market size from their relative live weights after about 10 months of growth. At both sites we have found significant differences in live weights of families, with some families weighing almost twice as much as other families at the Puget Sound site. Six families were ranked among the top 15 families of both sites, indicating that these families performed well at both sites, despite environmental differences.

THE MEDITERRANEAN MUSSEL (*MYTILUS EDULIS GALLOPROVINCIALIS*) IN PUGET SOUND AND COASTAL AREAS. Sean E. Matson, School of Fisheries, WH-10, University of Washington, Seattle, WA 98195.

The Mediterranean mussel (*Mytilus edulis galloprovincialis*) is currently cultured in Puget Sound in addition to the native mussel (*Mytilus edulis trossulus*). Massive mortalities common in the native mussel due to hemic neoplasia have not been observed in the Mediterranean mussel. The Mediterranean mussel also yields much more meat per individual. These factors make it valuable for culture. Controversy exists whether the native mussel will be displaced by the Mediterranean mussel either by out competing for physical space or by extensive hybridization. Previously, *galloprovincialis* has been found in embayments of high maximum annual sea surface temperature, where salinity is high and fluctuates little. Environmental and temporal factors limit the reproduction, hybridization, and distribution of these two sibling species. The two spawn at different times of the year with some overlap.

This study will document the distribution of the Mediterranean mussel and its hybrids in Puget Sound using allozyme electrophoresis. Sites in Oregon, California, and British Columbia will also be sampled to allow data comparison to previous studies. The PGM-2 locus is diagnostic in distinguishing these two mussels and their hybrids. This locus and four to six other loci known to be valuable in identifying these sibling species will be used to determine the proportions of each mussel and their hybrids in each sampled population. These proportions will be related to environmental factors such as minimum and maximum annual sea surface temperatures, salinity, site substrate characteristics, proximity of aquaculture operations, and net surface current dynamics.

MODELING THE GROWTH OF BOTTOM CULTIVATED SHELLFISH: A PRACTICAL APPROACH. Carter R. Newell, Great Eastern Mussel Farms, Inc., P.O. Box 141, Tenants Harbor, ME 04860.

In order to increase seed to harvest yields and reduce the grow-out period for bottom cultivated shellfish such as mussels and oysters, a modeling approach was used to establish the growth vs density relationships for individual aquaculture lease sites (Campbell and Newell, 1997; Newell, Campbell and Gallagher, 1997 JEMBE in press) in Maine, USA. A step-by-step methodology requires the following input data in order to characterize the supply and demand of food at the sites:

1. Current speed and direction from a finite-difference flow model.
2. Annual curves of surface particulate phytoplankton and detrital carbon and nitrogen around the period of high tide at the edge of the lease site.
3. Water depth, temperature and total particulate matter (SPM).
4. Shellfish filtration rates (demand).

5. Absorption efficiency of phytoplankton and detritus.
6. Physiological rate equations for the species of interest.
7. Settling rates of natural particulates at the site.

Using a model developed specifically for mussels (MUSMOD©), seeding densities were adjusted at three Maine farms to reduce grow-out time by 50% and increase seed to harvest yields. Application of the model to oysters in west coast waters will also require an analysis of density-dependent growth within culture units (i.e. oyster bags) as well as larger-scale effects of seston depletion over the lease site. An appreciation of the importance of small-scale (i.e. 50–100m) differences in current speed within lease sites will allow for better utilization of those sites if seeding densities are adjusted accordingly.

APPLICATION OF FLOATING UPWELLING SYSTEM (FLUPSY) FOR IMPROVED SURVIVAL AND GROWTH OF PACIFIC OYSTER SEED (*CRASSOSTREA GIGAS*) IN ALASKA. Raymond L. Ralonde, University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, Marine Advisory Program, 2221 E. Northern Lights Blvd. #110, Anchorage, AK 99508.

Alaska regulations allow only Pacific oysters *Crassostrea gigas* less than 20 mm in shell length to be imported into the state for aquaculture. The influences of Alaska's northern latitude and small size of the Pacific oyster seed cause high variability in growth and survival that severely hampers expansion of the oyster farming industry. Application of floating upwelling system (FLUPSY) nursery technology was tested on 5 mm Pacific oyster seed with the objectives to evaluate changes in seed survival and growth, develop improvements in FLUPSY design and operation, and measure the economic impacts of nursery culture on oyster farm operation.

With application of FLUPSY nursery technology farmers will experience a 10–30% reduction in mortality, improved uniformity in growth, and an estimated 40% reduction in farm construction and operation costs. Reduced variability of growth and survival improves production inventory estimates and access to larger, more secure markets. Continuing research is directed toward developing and refining design criteria and operation protocols for efficient application of FLUPSY nursery technology.

PHYSICAL AND BIOLOGICAL IMPACTS ON GROWTH AND MORTALITY OF *CRASSOSTREA GIGAS*. Jennifer L. Ruesink, Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver, B.C. V6T 1Z4.

What limits the spread of introduced Pacific oysters? On the west coast of North America, *Crassostrea gigas* rarely occurs outside of warm, protected bays, and ranges may be constrained by physical and/or biological factors that change over a gradient of wave exposure. In Barkley Sound, Vancouver Island, the presence

of oysters is inversely related to the abundances of strongly-interacting intertidal species. I transplanted spat to wave protected and exposed areas and performed a 2×2 factorial experiment manipulating competitors and predators. In early summer 1997, growth and survival were higher at protected than at exposed sites. Predator exclusion improved survival of oysters, but removal of competitors actually reduced survival. Microhabitat appeared extremely important for oyster growth, as all spat on a single piece of cultch tended to be affected similarly.

INVESTIGATIONS USING OYSTER CONDITION INDEX TO MONITOR THE AQUATIC ENVIRONMENT OF WILLAPA BAY WASHINGTON. Ervin J. Schumacker, School of Fisheries, University of Washington, Box 357980, Seattle, WA 98195, Brett R. Dumbauld and Bruce E. Kauffman, Washington Dept. of Fish and Wildlife, PO Box 190, Ocean Park, WA 98640.

Natural set and hatchery reared Pacific oysters, *Crassostrea gigas*, were transplanted to two sites within and just outside the mouth of the Willapa River. Samples from each of the four groups were taken monthly and oyster condition index (CI) determined on individual oysters using a gravimetric method and the Westley volumetric method which has been used by the Washington Department of Fish and Wildlife (WDFW) since the early 1960's.

Findings from this study have shown that the gravimetric and Westley volumetric methods are linearly correlated when performed on the same oysters and that the less time consuming and more precise gravimetric method can be used as an accurate gauge of oyster CI.

Relationships between oyster CI and short-term and long-term variations in aquatic conditions such as temperature, salinity, chlorophyll *a* content, and nutrient levels are being determined through ANOVA and multiple regression. Placement of the oyster test sites

was also done to monitor watershed activities (esp. storm events) and their effects on oyster CI.

Goals for this investigation include the standardization of the methods for determining oyster CI the use of this index as an indicator of conditions and trends in an estuarine environment, and determining if responses differ for hatchery reared and natural set oysters.

THE EUROPEAN GREEN CRAB IN OREGON. Sylvia Yamada, G. Allison, K. Cleveland, and C. Hunt, Zoology Department, Oregon State University, Corvallis, Oregon 97330, N. Richmond and J. Schaefer, Oregon Department of Fish and Wildlife, Box 5430 Charleston, Oregon 97420.

The European Green Crab (*Carcinus maenas*) has invaded and become established on several coastlines outside its natural range. Because this species can be an important predator, establishment of the green crab can cause serious changes in invaded communities such as displacement of endemic species through competition, dramatic reduction of prey populations and economic hardship on local aquaculture industries.

In 1989–1990, the green crab was discovered in San Francisco Bay and since spread south into Monterey Bay, and is now established north in Humboldt Bay. In March 1997, the green crab was discovered in Coos Bay, Oregon. Biologists and aquaculturists are very concerned that this crab will spread throughout Oregon, Washington and British Columbia.

We are monitoring this invasion-in-progress by collecting baseline data on the population structure of native crab and prey species in Coos, Winchester, Alsea and Yaquina Bay. We predict that the predatory and competitive effects of the green crab will be most noticeable in low salinity estuaries because this niche presently is not occupied by an efficient crab predator.

ABSTRACTS OF TECHNICAL PAPERS

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HYDROSTATIC PRESSURE OPENING OF OYSTERS, A NEW TOOL TO SHUCK AND KILL BACTERIA SIMULTANEOUSLY? Haejung An, Osu-Seafood Laboratory, 2001 Marine Dr., Rm 253 Astoria, Or 97103-3427.

High hydrostatic pressure (HHP) treatment has received a wide range of attention because of its effect on microorganisms and enzymatic activity, the two most detrimental factors to deteriorate quality and accelerate spoilage of foods. HHP treatment applied to fresh oysters has shown promising results by reducing the number of aerobic bacteria at medium pressure range and by opening shells at relatively low pressure range. With the advancement of technology, it may be possible to apply the HHP treatment in processing fresh oysters in commercial operations, and its potential effects on extending shelf life, reducing pathogens, such as naturally present *Vibrios* in fresh oysters and opening of live oyster shells will be discussed.

SETTLEMENT BEHAVIOR OF DUNGENESS CRAB, *CANCER MAGISTER*, MEGALOPAE WHEN EXPOSED TO CHEMICAL AND VISUAL CUES FROM THE SHORE CRAB, *HEMIGRAPUS*. Janel L. Banks, Undergraduate Environmental Science, Huxley College, Western Washington University, Washington 98225, Dr. Paul A. Dinnel, Shannon Point Marine Center, Anacortes, Washington, 98221.

Artificial habitat is often created to augment populations of commercially valuable species. The complex structure of the habitat serves to provide refuge from predation. In Grays Harbor, WA, this concept is being applied by establishing intertidal oyster shell plots for Dungeness megalopae settlement. Previous monitoring of these plots revealed a negative relationship between *Hemigrapsus* populations and Dungeness crab juveniles within the shell plots. The present study investigated the relationship between the presence of *Hemigrapsus* and the settling behavior of Dungeness megalopae. The study also explores the predation interactions between *Hemigrapsus* and Dungeness crab instars.

Dungeness crab megalopae avoid the vicinity of *Hemigrapsus*, whose presence is detected chemically, not visually. Results support the hypothesis that Dungeness megalopae are avoiding the presence of *Hemigrapsus* when selecting areas to settle. *Hemigrapsus* predation upon Dungeness crab, with carapace width of 8 cm, begins to occur when the shore crab is approximately 1.5 times the size of the Dungeness. Percentage mortality of Dungeness instars by predation increases as the size ratio (Dungeness crab: *Hemigrapsus*) increases.

IS THE LABORATORY PERFORMANCE OF PACIFIC OYSTER SPAT, *CRASSOSTREA GIGAS*, EXPOSED TO VARYING SALINITIES, PREDICTIVE OF PERFORMANCE IN THE FIELD? Chris Brooks and Chris Langdon, Oregon State University, Hatfield Marine Science Center, 2030 S. Marine Science Drive, Newport, OR 97365.

Broodstock selection of oysters is a lengthy process which may take up to three years. In an effort to study the feasibility of shortening this time period, 72 representative oysters from each of the 14 full-sib families available were individually tagged as 7-10 mm spat. Commercially available Kumamoto, triploid and tetraploid oysters were included for comparison. Spat were placed into uncrowded upwellers and fed to excess on a diet of *Chaetoceros calcitrans*, and *Isochrysis galbana*. Whole wet weights and survival were measured approximately bi-monthly for 11 months. Half of the oysters were exposed to increasingly severe (i.e., 30ppt–5ppt) salinity fluctuations that simulated typical winter tide cycles and a 1996 storm event in Oregon's Yaquina Bay. The pre-flood family rankings of specific growth rate and survival were similar between fluctuating and constant salinity conditions, based upon Spearman Rank Correlation, ($\rho = 0.893, 0.833$ respectively). Mean family SGR values ranged from 4.56% to 1.3%. The five families with the highest pre-flood mean SGR were the tetraploids, followed by diploid families #106, #108, #105 and the triploids. Results of the family rankings in the laboratory environments compared with their rankings at two different grow-out sites in the Yaquina Bay estuary will be presented.

OYSTER SUMMER MORTALITY—AN UPDATE ON ONGOING SEA GRANT SPONSORED RESEARCH. D. P. Cheney, R. Elston, and B. MacDonald, Pacific Shellfish Institute, 120 State Ave. N.E. #142, Olympia, WA 98501-0600 and PO Box 687, Carlsborg, WA 98324.

While oyster production on the U.S. west coast has not experienced the catastrophic losses from disease plaguing the east coast, mass mortalities periodically occur on commercial farms. Pacific oysters (*Crassostrea gigas*) from growing areas in Washington, Oregon and California sporadically experience larger than expected die-offs in summer months. Sharp increases in mortality from Puget Sound, Washington from June to September are considered the classical example of west coast summer mortality. More recently, losses of seed and adult oysters in Europe have also been attributed to summer mortality.

Work began in early 1998 on a Sea Grant Program funded study to more precisely characterize summer mortality in a variety of culture conditions and locations, definitively describe the relationship to infectious diseases, and identify water quality and sea-

sonal patterns. Also, underway is a field component to investigations into the oyster thermal stress response and an assessment of induced thermal tolerance to reduce mortalities. In addition, management practices for commercial cultivation are being evaluated as possible measures to reduce the frequency and extent of oyster losses.

Ambient environmental conditions monitored to date indicate oysters are subject to extreme variations in a number of parameters during intertidal cycles. Peak temperatures neared 53°C during exposure at low tide, and dissolved oxygen levels were below 3 mg/l under neap tide conditions. Mortalities occurred in all size groups and under varying culture conditions. Triploid oysters had a cumulative mortality rate of up to 2 1/2 times diploid oysters planted at the same density. Preliminary histological analysis indicated changes which could result in metabolic stress and clinically observed gaping and weak shell closure. Expression of thermal stress proteins occurred in animals from all sampled stations. Further evaluation of these results and additional field research is underway.

PSP IN GEODUCKS: VARIABILITY, ANATOMICAL DISTRIBUTION, AND COMPARISON OF TWO TOXICITY TESTING METHODS. Kelly M. Curtis, University of Washington, School of Fisheries, Box 357980, Seattle, WA 98195.

The purposes of this study were to: (1) determine variability in paralytic shellfish poisoning (PSP) toxicity in the geoduck clam (*Panope abrupta*), i.e., within a single population, between various sets of populations, and seasonally; (2) determine anatomical distribution of PSP toxins; and (3) compare two PSP testing methods. From summer through winter 1997, 15–20 geoducks were collected biweekly from a shallow and a deep location in each of two tracts in Puget Sound, Washington: Agate Pass (AP) and Quarter-master Harbor (QH). Geoducks were dissected into siphon, mantle, and visceral portions. All portions were assayed separately using the mouse bioassay (MBA), while only the visceral portions were assayed using the receptor binding assay (RBA).

Results indicated that individual variability was high in the shallow areas with coefficients of variation (CV) ranging from 20–98%, and lower in the deep areas (CV = 18–62%). Seasonally, variability did not change in either of the QH areas and increased in both of the AP areas. Only shallow geoducks were toxic in QH. All geoducks were toxic in AP, with the shallow ones significantly more toxic 18% of the time. Anatomically, PSP toxins were isolated to the visceral ball in all geoducks throughout the study period. There was a highly significant positive correlation between the MBA and RBA, with the RBA slightly overestimating the MBA at lower toxicity levels.

Variability information will aid fishery managers in maximizing utilization of the resource, while at the same time protecting public health from the dangers of PSP.

INTENSIVE BIVALVE HEALTH MANAGEMENT. Ralph Elston, Pacific Shellfish Institute, PO Box 687, Carlsborg, WA 98324. Jerry Heidel, Oregon State University, Corvallis, OR. Joth Davis, Taylor Resources, Shelton, WA, and Dan Cheney, Pacific Shellfish Institute, Olympia, WA.

Growout of edible shellfish is practiced on a more or less extensive basis, but intensive hatchery production is now an essential part of culture. Intensification brings new challenges for health and disease management including a variety of highly opportunistic and aggressive infectious agents as well as nutritional and genetic diseases, and toxic conditions.

Infectious diseases that have emerged in intensive hatchery and nursery operations include velar virus disease, herpes virus disease, vibriosis, hinge ligament disease, acute and chronic extapallial bacterial infections, bacterial mat disease, ameboflagellate disease and invasive ciliate disease. A recent example are bacterial mats resulting in debilitating infections of juvenile geoducks, *Panope abrupta*. These infections are initiated by Cytophaga-like bacteria that colonize the external shell surface and subsequently invade the mantle of juvenile clams, necrotizing the entire mantle. The bacterial mats may interact with the texture of composition of juvenile geoduck shells since this condition has not been observed in any other species.

These conditions can be managed and ameliorated using principles of responsive health management. In many cases, there is a great deal of information lacking that is needed to fully accomplish the goal of maintaining healthy cultures of shellfish in the hatchery, nursery and in grow out areas. Sanitation and health management are two distinct objectives of intensive bivalve high health program that will be discussed. Both objectives should be met by identifying standards, monitoring and responding to deviations in sanitary or health and condition standards.

Supported in part by NMFS Saltonstall Kennedy Program and USDA Small Business Innovative Research Program.

ECONOMIC FEASIBILITY OF CO-CULTURING RED ABALONE *HALIOTIS RUFESCENS* WITH RED MACROALGAE *PALMARIA MOLLIS* AT TWO GEOGRAPHICALLY DISTINCT LOCATIONS. Ford Evans and Chris Langdon, Oregon State University, Hatfield Marine Science Center, 2030 S. Marine Science Dr., Newport, Oregon 97365.

The abalone industry on the west coast of the United States relies primarily on the harvest of wild macroalgae as a food source. Unpredictable weather events and strict governmental regulations may limit the availability of these algae to aquaculturists in the future. Recent studies have shown co-culture of red abalone (*Haliotis rufescens*) with the red macroalga dulse (*Palmaria mollis*) to be an effective method of abalone production. When co-cultured with abalone, dulse is a nutritious food source as well as an in situ biofilter. In addition, the producer can control dulse quantity and quality at the farm site.

Data from these previous studies allowed the development of a bioeconomic model for abalone/dulse co-culture. This paper examines a series of enterprise budgets for hypothetical co-culture farms located in Oregon and Hawaii. These farms were hatchery/nursery operations which grew 500,000 abalone to 20 mm shell length. The farms in Hawaii and Oregon were identical, apart from different management practices and environmental conditions.

Results showed abalone were, in general, cheaper to produce in Hawaii than in Oregon. This was primarily due to the fact that artificial lights were required in Oregon to achieve the light intensities that occur naturally in Hawaii. The use of an artificial diet to supplement dulse as a food source reduced costs in all treatments. The model further indicated that juvenile abalone could be grown economically in Oregon over winter with the use of both supplemental illumination and a diet consisting of a combination of dulse and artificial feed.

THE GROWTH OF BUTTER CLAMS (*SAXIDOMUS GIGANTEUS*) ON SELECTED BEACHES IN THE STATE OF WASHINGTON. Stuart A. Goong and Kenneth K. Chew, School of Fisheries, University of Washington, PO Box 357980, Seattle, WA 98195.

The butter clam is among the most valuable species for recreational shellfish harvesters, but is currently of little commercial importance in Washington. Recently, a small commercial harvest began for the clam, and commercial interest is expected to increase. Basic information on the biology of the clam is necessary to establish a sound management regime.

The primary objective of this study is to examine the growth rates of butter clams on three beaches in the Puget Sound Basin and to determine whether these rates may differ. If growth rates are significantly different, then a more localized management strategy may be required to protect the productivity of the stock. Otherwise, a more generalized management approach may be adequate. The beaches included in this study are Birch Bay State Park, Double Bluffs Beach, and Potlatch State Park.

Growth rates were determined by measuring lengths-at-age for clams collected from each beach. Using a second order linear regression model, growth rates were found to be significantly different, with the fastest growth occurring at Double Bluffs Beach, followed by Birch Bay. The growth rate at Potlatch was slowest, although this was determined from a relatively small sample of animals. Von Bertalanffy growth curves produced using nonlinear regression yielded similar results. Clams in the 3+ age class are expected to reach a length of 43 mm at Double Bluffs, 40 mm at Birch Bay, and 35 mm at Potlatch. In the 2+ age class, only clams from Double Bluffs are expected to reach a legally harvestable size of 33 mm.

THE VARNISH CLAM (*NUTTALLIA OBSCURATA*): ANOTHER COMMERCIAL SPECIES? William A. Heath, BC Fisheries Ministry, Seafood Development Section, 2500 Cliffe Avenue, Courtenay, BC Canada V9N 5M6.

Originally from Japan, the varnish or dark mahogany-clam (*Nuttallia obscurata*), was unintentionally introduced to the Strait of Georgia, BC during the 1980s–1990s. This attractive-looking clam reaches sizes to 2.25" (55 mm) and is found mainly in sand-gravel substrate to 8" (20 cm) in the high to mid-intertidal zone. At present, quantitative data on its distribution are limited, but further sampling is in progress. Preliminary data indicates that this exotic species is widely distributed on beaches in the Strait of Georgia, but it has also been reported in Puget Sound, Washington and in Barkley Sound, BC. Commercial concentrations have been found on some clam tenures in Baynes Sound, BC. A small quantity of product was test marketed recently, with favourable response from food services customers regarding appearance, meat:shell ratio and shelf-life characteristics. Due to food safety concerns related to marine biotoxin (PSP) retention times, further harvest has been curtailed in BC until sufficient data are available to shellfish safety agencies (e.g., Canadian Food Inspection Agency) to determine appropriate safety protocols. Preliminary data indicate similarities with levels of PSP found in Manila clams and Pacific oysters during a toxic bloom in Okeover Arm, BC. Provided that safety issues are resolved satisfactorily, the Ministry of Fisheries is prepared to approve the licensing of varnish clam culture on shellfish tenures. Harvest of this species from untenured areas will be subject to Department of Fisheries and Oceans policy considerations. The potential for developing this introduced species into a valuable shellfish product appears to be very high, providing an exciting new opportunity to diversify the shellfish industry.

CROSSBREEDING TO IMPROVE PACIFIC OYSTER BROODSTOCKS: UPDATE OF USDA-WRA PROJECT. Dennis Hedgecock, University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94952-0247.

This collaborative project has four major objectives: (1) to test the performance of hybrids made by crossing inbred lines, at a commercial scale, (2) to make new inbred lines from select pedigreed families in Oregon State University's Molluscan Broodstock Program, (3) to synthesize triploids and eventually tetraploids from WRAC inbred lines and test their performance, and (4) to enable early detection of metabolic potential for growth by determining the metabolic basis of hybrid vigor. Four hybrid spawns have been carried out at the Taylor Resources, Inc., hatchery on Dabob Bay, each yielding 8–10 million seed, which have now been planted in Thorndyke and Samish Bays. In the hatchery, two mixed-hybrid groups grew faster and settle 2–7 days earlier than larvae from typical commercial spawns. DNA markers will be used at harvest to determine the relative performances of different hybrids in these mixed groups and thus the best inbred lines for further crossbreed-

ing. DNA markers are also being used to map genes causing growth heterosis in F2 and F3 hybrid populations. New inbred lines were initiated by sib-mating within the nine, top-performing MBP families grown in Tomales Bay. Seventeen triploid groups have been synthesized with one-, two-, and three-way combinations of inbred-line genomes. Inbred triploids have the slowest larval growth, but differences between triploids made from reciprocal hybrid females are sometimes evident (i.e., AB does not equal BA). Triploids are being reared to provide broodstock for tetraploid induction. Finally, of the 13 physiological traits compared in inbred and hybrid larvae, most in multiple experiments, six show no relationship to growth heterosis (total protein, mitochondrial density, lipid content, assimilation rate, shell growth, and carbonic anhydrase activity). Four factors (respiration and feeding rates, sodium ion pump activity and protein synthesis) show a relationship to heterosis in some but not all experiments, while three factors (embryo respiration rates and whole-body and specific protein turnover rates) show a consistent relationship with growth heterosis. The growth advantage of hybrids appears to be based on a greater metabolic efficiency, with lower rates of protein turnover and respiration. Dramatic differences in respiration rate are evident as early as 15 hrs post-fertilization, suggesting that hybrids "get out of the starting blocks early." Two-dimensional gel-electrophoretic analyses show that a few, small, acidic proteins are responsible for most of the inbred vs. hybrid difference in whole-body protein turnover. These proteins can be sequenced and their genes mapped.

THE ARRIVAL OF THE EUROPEAN GREEN CRAB, *CARCINUS MAENAS*, IN OREGON ESTUARIES. Chris Hunt and Sylvia Behrens Yamada, Zoology Department, Oregon State University, Corvallis, Oregon 97331-2914 and Neil Richmond, Oregon Department of Fish and Wildlife, P.O. Box 5430, Charleston, Oregon 97420.

Since its first discovery in Coos Bay, OR in 1997, *Carcinus maenas*, is now found in at least seven Oregon estuaries: Coos, Alsea, Yaquina, Siletz, Salmon, Netarts and Tillamook. All of the crabs found in Coos Bay in 1997 were large crabs, ranging in size from 54–86 mm CW (carapace width). We estimate that they represent the 1995/1996 year class. Similar sized crabs were found in Tillamook and Netarts Bays this year. During the summer of 1998, a new year class appeared in Oregon estuaries as well as in Humboldt Bay, CA to the south and Willapa Bay and Grays Harbor, WA to the north. These crabs averaged 14 mm CW in June, 27 mm in July and 48 mm in August. This coast-wide colonization event is correlated with unusually strong northward moving coastal currents off the Oregon coast from September 1997 to spring of 1998. Transport of larvae from well established populations to the south, rather than oyster transport, appears to be the mechanism for the appearance of this new year class. This research was supported by Oregon Sea Grant.

PERFORMANCE OF GALLO MUSSELS FROM DIFFERENT SOURCE POPULATIONS. Kurt Johnson, Taylor Resources, Inc., 701 Broadspit Road, Quilcene, WA 98376.

Taylor Resources Inc. operates commercial mussel farms using *Mytilus edulis galloprovincialis* (Gallo) mussels at different sites in Puget Sound. Seed for these farms is produced in the Taylor Resources Hatchery. We have the opportunity to improve the performance of mussels through the control of brood stock. This study is a beginning point in our brood stock program by evaluating the performance of different mussel populations. We also looked at the performance of diploid versus triploid mussels. Brood stock Gallo mussels were collected from the Totten Inlet farm selected survivors from the Holmes Harbor farm, Dyes Inlet wild populations and Sequim Bay wild populations. Seed mussels were raised in the hatchery, planted on lines in Holmes Harbor, then transferred to oyster bags for final grow out. During final grow out the populations were sampled for survivorship and weight. At the end of the study differences were evident in survival production and size among some of the populations and between diploids and triploids. The results suggest that the source population of brood stock and ploidy is important in farm performance of the mussels. The results also lead to questions about the genetic differences of Puget Sound Gallo stocks, their origins and length of time as Puget Sound residents.

UPDATE ON THE MOLLUSCAN BROODSTOCK PROGRAM. Chris Langdon, Coastal Oregon Marine Experiment Station, Hatfield Marine Science Center, Oregon State University.

The Molluscan Broodstock Program (MBP) focuses on improving commercial oyster production on the West Coast by both genetic selection and development of effective broodstock management strategies. Researchers at Oregon State University (OSU) together with industry partners have implemented a selective breeding scheme for Pacific oysters that involves comparing the performance of full-sib families planted at different commercial sites on the West coast. Selection of superior families for production of subsequent generations should result in long-term improvement in commercial oyster yields.

Since MBP's inception in 1995, about 250 oyster families have been planted at commercial sites in Alaska, California, Oregon and Washington. Significant differences in family performance have been observed with top performing families yielding about twice the meat yields per bag compared with the poorest families, suggesting excellent potential for genetic improvement.

In spring 1998, we crossed the top performing families from Hog Island, Tomales Bay, California, to produce a second generation that was planted with Hog Island in the fall. We will compare

the performance of these families with that of families derived by crossing unselected "wild" oysters as well as oysters produced by industry.

Genetic "finger-printing" of broodstock oysters used in the MBP breeding program ensures that pedigrees are correct contamination of subsequent MBP generations is avoided.

LARVAL SURVIVAL AND GROWTH OF MYTILUS TROSSULUS AND M. GALLOPROVINCIALIS HYBRIDS. Sean E. Matson, J. P. Davis, and K. K. Chew, School of Fisheries, University of Washington, Seattle, WA 98195.

Experiments were performed to determine whether survival and growth differences existed between the larvae of two locally occurring species of marine mussels and their reciprocal hybrids at two different salinities. The two species used were *Mytilus trossulus*, the Baltic mussel, and *M. galloprovincialis*, the Mediterranean mussel. Preliminary results are presented.

Broodstock were collected from essentially monospecific populations and were screened morphologically. They were positively identified at two DNA loci using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques.

The four crosses were *M. trossulus* × *trossulus* (TxT), *trossulus* female × *galloprovincialis* male (TxG), *galloprovincialis* female × *trossulus* male (GxT), and *galloprovincialis* × *galloprovincialis* (GxG). The low salinity treatment was 20 parts per thousand (ppt) and the high salinity was 30ppt. The low salinity treatment was applied after three days.

Survival at day three was analyzed as arcsine transformed percents using one-factor analysis of variance (ANOVA). The TxT cross survived significantly better than the other three crosses at day three ($p < 0.05$). Size at age was measured as shell length in microns and analyzed using two-factor ANOVA. At day 14, the mean shell length of all crosses differed significantly except TxG and GxG ($p < 0.05$). These were smaller than the other two crosses. The mean size of low salinity cultures was significantly larger than that of high salinity cultures. Different crosses reacted differently to salinity ($p < 0.05$). This was because mean size for the GxG cross was nearly equal between high and low salinities.

GREEN CRABS AND NATIVE PREDATORS: POSSIBLE LIMITATIONS ON THE WEST COAST INVASION. P. Sean McDonald, Gregory C. Jensen, and David A. Armstrong, School of Fisheries, University of Washington, Seattle, Washington 98195.

The European green crab, *Carcinus maenas*, is a voracious predator and aggressive colonizer. Introduction of the species to West Coast estuaries and subsequent rapid range expansion have led to predictions of impact on native species and local aquaculture.

We conducted a survey of established populations of *Carcinus* and native crab in Bodega Harbor, California. A recruitment experiment was initiated in an intertidal oyster shell habitat. The results of the study are synthesized with recent data from Washington estuaries and significant historical literature concerning *Carcinus*. Observations of juvenile and adult *Carcinus* behavior were also undertaken.

Preliminary data suggests that native West Coast predators and competitors may restrict *Carcinus* distribution and impact. West Coast populations appear to be limited to *Spartina* beds, high intertidal areas, and low-salinity refuges. Incidence of limb autotomy in Bodega *Carcinus* occupying areas inhabited by *Cancer productus* and *C. antennarius* was significantly higher (81%) than in crabs found in areas free of *Cancer* spp. (44%) or European populations (42%). Recruitment of *Carcinus* into intertidal oyster shell habitat may be limited by *Hemigrapsus oregonensis*. In experimental shell plots monitored for six months, *Carcinus* density was less than 6 crabs · m⁻² while *Hemigrapsus* density increased to nearly 1000 crabs · m⁻². Potential interactions with other Pacific Northwest predators are discussed, as well as areas for future investigation.

REVIEW OF STUDENT STUDIES ON JUVENILE GEODUCKS AT MALASPINA UNIVERSITY COLLEGE'S FISHERIES AND AQUACULTURE PROGRAM. Melissa Milne and Jesse Ketter, Fisheries and Aquaculture Diploma Program at Malaspina University College.

Since 1994 a series of studies have been conducted by the Fisheries and Aquaculture Program at Malaspina University/College on the Pacific Geoduck, *Panopa abrupta*. These studies, supported by Fan Seafoods Ltd, have included: morphometric relationships, orientation and digging behaviour, effects of crab predation, salinity tolerance tests, and most recently a study of moon snails as predators of juvenile geoducks.

The first moon snail study was designed to assess predation mode and rate by the Arctic moon snail, *Cryptonatica affinis*, on juvenile geoducks. The results indicated that Arctic moon snails (shell diameter 28–30 mm) did prey on geoducks (28–30 mm shell diameter) at a rate of 1.5–2.5 geoducks per moon snail/week. The snails either bored into the geoduck shell or apparently digested the entire juvenile without boring.

A second study assessed the Lewis's moon snail, *Polinices lewisii*, as a predator of juvenile geoducks and was also designed to compare food preference between geoducks and the Manila clam, *Tapes philippinarum*. The moon snails used averaged 103 mm shell length, the manila clams 30–50 mm, and the geoducks 15–21 mm. The observed predation rates were very low for both species of clams and further trials are needed to clarify the importance of the Lewis's moon snail for geoduck aquaculture.

WATER QUALITY, SHELLFISH AND PUBLIC HEALTH IN TOMALES BAY, CALIFORNIA. Paul G. Olin, University of California Sea Grant Extension, Santa Rosa, CA 95403, Gregg Langlois, California Department of Health Services, Berkeley, CA 94704.

In October of 1993, the California State Legislature passed the Shellfish Protection Act to protect water quality in shellfish growing areas. The ACT mandated the Regional Water Quality Control Board to assemble a technical advisory committee whenever a commercial shellfish growing area is threatened to identify and remediate pollution sources. In January of 1994, the Tomales Bay Shellfish Technical Advisory Committee was formed and in 1995 a study was conducted to identify potential pollution sources.

The results of the study indicated that significant coliform inputs were derived from surface waters flowing into the Bay. Potential sources of coliforms in the Bay are manure from livestock and dairy cattle, wildlife scat and human sewage entering the Bay via faulty septic systems for from boaters.

In May of 1998 there was an outbreak of food borne illness associated with consumption of raw oysters that was traced to a viral pathogen unique to humans. This resulted in a closure of Bay waters to shellfish harvest for 26 to 81 days. This outbreak clearly demonstrates the need to aggressively monitor septic systems. This recommendation was made by the Shellfish Technical Advisory Committee in 1994 and was not acted on. The chronology of this outbreak will be presented along with a discussion of stepped up efforts to prevent a reoccurrence.

A SPRAY-DRIED *SCHIZOCHYTRIUM*-BASED DIET CAN COMPLETELY REPLACE LIVING ALGAE FOR REARING JUVENILE MUSSELS (*MYTILUS GALLOPROVINCIALIS*). Ebru Onal and Chris Langdon, Coastal Oregon Marine Experiment Station and Department of Fisheries and Wildlife, Hatfield Marine Science Center, Oregon State University.

Growth experiments were conducted with juvenile (0.04–0.21 g live wt.) mussels (*Mytilus galloprovincialis*) fed on a diet of living algal cells [a mixture of *Chaetoceros* sp. (CHAET) and *Isochrysis galbana* (Tahitian strain; T-ISO)] either partially or completely substituted with a spray-dried diet based on *Schizochytrium* sp. (SCHIZO; Docosa Gold, Sanders Brine Shrimp Company, Utah).

Preliminary experiments with mussels indicated that combination of SCHIZO and *Spirulina* (= *Arthrospira*) *platensis* (SPIRO; Spirulina 50, Sanders Brine Shrimp Company, Utah) was a promising substitute for living algal diets. Subsequently, we found that a full ration of algae (1:1 by cell number CHAET and TISO) could

be completely substituted with an equivalent dry weight of 1:1 (by wt.) mixture of SCHIZO and SPIRO with no significant (Scheffe test; $p > 0.05$) adverse effect on final live weights.

Furthermore, growth of mussels fed on living algal rations could be significantly (Scheffe test; $p < 0.05$) improved with supplements of either a 1:1 (by wt.) mixture of SCHIZO and SPIRO or SCHIZO alone.

IDENTIFICATION AND TRACKING OF THE SOURCES OF MICROBIAL POLLUTION IN SHELLFISH GROWING WATERS. Mansour Samadpour, Assistant professor, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195.

The lack of appropriate methodology for tracing bacterial contamination in the environment is a major impediment in identification and control of the sources of these pollutants and adversely affects the decision-making process in water quality management, and management of fisheries resources. Several methods for tracking genetically engineered microorganisms have been used, but their utility is limited to the detection of organisms carrying reporter genes or their products. Limited efforts to track sources of natural bacterial populations have been made, the approach used was based on quantitation of indicator organisms at various sites. These studies invariably have resulted in raising more questions rather than providing answers. I have developed and tested a tracking system for identification of sources of microbial pollution. The methodology can be used to assess the impact and contribution of point and non-point sources of fecal pollution (animals, septic tanks, farms, sewage treatment plants, etc.) on the fecal coliform level in shellfish growing waters. It can also be used to map the distribution, transportation, and movement of indicator organisms and microbial pathogens in watersheds and water.

The Ribo-Tracking method has been used to determine the sources of fecal coliforms in a variety of settings. Results of one of the recent studies involving shellfish growing areas in Grays Harbor, Washington will be presented and discussed.

RELATING OYSTER CONDITION INDEX TO THE AQUATIC ENVIRONMENT OF WILLAPA BAY WASHINGTON. Ervin J. Schumacker, School of Fisheries, University of Washington, PO Box 357980, Seattle, WA 98195, Brett R. Dumbauld and Bruce E. Kauffman, Washington Department of Fish and Wildlife, PO Box 190, Ocean Park, Washington 98640.

Natural set and hatchery reared Pacific oysters, *Crassostrea gigas*, were transplanted to two sites within and just outside the mouth of the Willapa River as it enters Willapa Bay. Samples from each of the four groups were taken monthly and oyster condition index (CI) determined on individual oysters using a gravimetric method, a volumetric method and a dry shell method.

Findings from this study have shown that the gravimetric method is linearly related to the volumetric method currently in use by the Washington Department of Fish and Wildlife and can be used as an accurate gauge of oyster CI with much less time and effort. The dry shell method may also be applicable with oysters of the same year-class.

Site differences for CI response are noted in the study and relationships between oyster CI and variations in environmental conditions such as temperature, salinity, chlorophyll a content, and sediment loads are determined by multivariate analysis.

Goals for this investigation include the recommendation of a standard method for determining oyster CI, the use of this index as an indicator of conditions and trends in an estuarine environment, and determining if hatchery reared and natural set oysters differ in their response to aquatic conditions.

INDUCTION OF TRIPLOIDY IN THE GEODUCK CLAM, *PANOPE ABRUPTA*. Brent A. Vadopalas, School of Fisheries, University of Washington, PO Box 357980, Seattle, Washington 98195. Jonathan P. Davis, Baywater Incorporated, 15425 Smoland Lane, Bainbridge Island, Washington 98110.

The development of geoduck culture techniques coupled with increased market demand led to cultured intertidal geoduck beds. Concerns then arose regarding the potential genetic risk posed by the reproductive contribution of hatchery outplants to wild stocks that may be genetically different. Although an ongoing study to determine the genetic stock structure of Puget Sound geoducks has yet to yield definitive results, the development of techniques to produce sterile triploids would enable geoduck culture to proceed irrespective of any genetic differences found. Moreover, triploid geoducks may exhibit an increased growth rate.

Geoduck eggs were fertilized and distributed among three temperature and three salinity treatments to test the effects of these factors on the timing and synchrony of meiotic events. Samples

were taken at 5 minute intervals to measure proportions of eggs at each meiotic stage. These data indicated a temperature and salinity combination for optimal triploidy induction of 15°C and 30 ppt., with the meiotic period between expulsion of the first and the second polar body lasting 20 minutes, beginning at 50 minutes post fertilization.

We also investigated two chemical treatments, cytochalasin B (CB) and 6-dimethylaminopurine (6-DMAP), to evaluate their suitability for triploid induction in geoducks. We found optimal triploid induction (>95%) resulted from a 600 M 6-DMAP treatment using our optima. Preliminary data indicates survivorship to straight hinge was about 20%. Survivorship to metamorphosis was highly variable among groups, which could not be attributed to a single factor. Surviving triploid geoducks have been outplanted to evaluate growth and survivorship.

GROWTH AND SURVIVAL OF INTRODUCED AND NATIVE BLUE MUSSELS (*MYTILUS* SPP.) IN GEORGIA STRAIT, BC: PROSPECTS FOR MUSSEL AQUACULTURE. Jenia Yanick and Daniel Heath.

Recently, permission was granted by the Ministry of Agriculture, Fisheries and Foods (now Ministry of Fisheries) to begin commercial rearing and harvesting of imported species of blue mussels (*Mytilus edulis* and *M. galloprovincialis*) in BC. Historically there has been little or no commercial harvest or culture in British Columbia due to the small size attained by the native blue mussel (*M. trossulus*). As it was unknown whether this influx of non-native mussels could become an environmental concern in Georgia Strait, it was proposed that an investigation be made into the feasibility of using mussels already present in Georgia Strait (native and non-native) as brood stock for future mussel culture. Presently mussels collected from Georgia Strait are being grown and monitored at Yellow Island Aquaculture Ltd. YIAL is an organic salmon farm interested in the potential of expanding into mussel aquaculture and is located on Quadra Island, BC.



ABSTRACTS OF TECHNICAL PAPERS

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TRYING TO COVER THE BASES: SEVEN YEARS EXPERIENCE OF ONE NEW YORK TOWN'S SHELLFISH ENHANCEMENT EFFORTS AND THE INCORPORATION OF AN OYSTER MARICULTURE TRAINING PROGRAM FOR COMMERCIAL FISHERMAN. J. Aldred and Craig Hassler, East Hampton Town Shellfish Hatchery, 159 Pantigo Road, East Hampton, NY 11937, G. Rivara, Cornell Cooperative Extension of Suffolk County Marine Program, 3690 Cedar Beach Road, Southold, NY 11971.

The eastern end of New York's Long Island has had its traditional marine resource based economy sorely tested in the past decade. There has been a near elimination of several of the most valuable fisheries due variously to Hudson River contaminants in fish, unusual smothering algae blooms locally known as brown tide, more stringent regulatory and water quality standards and competition from recreational interests. Relief has been sought partially through intensified resource enhancement efforts focusing on the shellfishery. The Town of East Hampton intensified its intermittent shellfish enhancement efforts in 1991 by instituting a hatchery based production program with a goal of distributing ten million seed shellfish onto public beds annually. Systems for brood stock conditioning, spawning, larval and post set culture, nursery and field grow out have been designed to integrate the production of oysters, hard clams and bay scallops to meet the desired needs of the community. Certain oyster and hard clam harvest returns showing strong hatchery influence have proven promising; nevertheless, the elevated expectations of some in the community has lead to the consideration of a more direct inclusion of fishing community members in an extension of the husbandry process. Through New York State and the F.I.G. program of NMFS and in cooperation with Cornell Cooperative Extension, twenty interested fishermen in the Town are participating in a pilot scale oyster culture training program, each receiving one hundred thousand seed oysters and the gear in which to grow them using simple off bottom culture techniques. In part, the program is attempting to encourage a coalescence of the public and private benefit of increased shellfish resources in the public domain.

FRACTAL DIMENSION AND COMPLEXITY OF OYSTER REEFS AS MODIFIERS OF FISH UTILIZATION AND SURVEYS OF REEF RESIDENTS. T. D. Alphin, R. J. Grifflitt, and M. H. Posey, Department of Biological Sciences, University of North Carolina at Wilmington, 601 S. College Road, Wilmington, NC 28403.

The utilization of structure by fish has been studied and reviewed in a number of papers from freshwater and marine systems. Within estuarine systems there has been less work done on the value of different structural habitats and on the factors that influence utilization of structural habitats. In southeastern North Caro-

lina, seagrasses (shown in other areas to be a preferred habitat type) are absent from the estuarine system, leaving oyster reefs as the major structural habitat in intertidal and shallow subtidal areas. We conducted surveys of oyster reefs in three tidal creeks systems to establish composition and abundance of non-fish species using this structure. This data was collected using pit traps. The second part of this study takes an initial look at how overall shape and complexity (fractal dimension) of an oyster reef influences the number of fish using it. Fish were sampled within the reef and two meters away from the edge of the reef (fish caught on the edge were presumed to utilize the oyster as a refuge to dart to while foraging on adjacent sand flats). Photographic analysis was used to determine fractal dimension and convolution of edge for five oyster reefs. No difference was detected in the total number of fish using a reef (within the reef and on the edge) regardless of fractal dimension. However, fractal dimension did have a significant effect on the proportion of fish foraging within a specific reef compared to immediately outside the reef.

ARE OYSTER REEFS ESSENTIAL FISH HABITAT? USE OF OYSTER REEFS BY ECOLOGICALLY AND COMMERCIALLY IMPORTANT SPECIES. D. Breitburg, The Academy of Natural Sciences, Estuarine Research Center, St. Leonard, MD 20685, T. Miller, University of Maryland, Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, MD 20688.

Are oyster reefs essential fish habitat (EFH)? The Magnuson-Stevens Fishery Conservation and Management Act defines EFH as "those waters and substrate necessary to fish (inclusive of finfish, shellfish, crustaceans and others) for spawning, breeding, feeding, and or growth to maturity." Oyster reefs certainly qualify as EFH under this definition because of their importance to the oysters themselves. However, a larger question remains as to whether oyster reefs can be considered 'essential' and necessary for finfish and crabs. If their importance to finfish and crabs justifies the designation of oyster reefs as EFH, we need to determine which kinds of fish and crabs justify the EFH designation and what characteristics of oyster reefs are required to enhance finfish and crab abundance, feeding and reproduction. Research at a number of sites along the Atlantic and Gulf of Mexico Coasts strongly indicates that there is a resident finfish assemblage that is dependent on oyster reef habitat. For these finfish species, the physical extent of oyster reefs, their suitability as refuges from predators and as sites for reproduction, and the level of production of invertebrate prey of finfish supported, are likely to affect abundance, growth and reproduction. In addition, the vertical relief of reefs can influence the oxygen environment to which resident fish are exposed, the delivery and distribution of larvae and the abundance of planktonic prey. A much larger number of finfish species and

blue crab utilize oyster reefs for feeding and, in some cases, shelter. We will use results of our ongoing study in the Patuxent River, as well as those of other researchers, to discuss the importance of oyster reefs to these species that are more loosely associated with oyster reefs, the importance of physical scale of restoration efforts, and the potential importance of the spatial distribution of reefs in the estuarine landscape.

MAKING A CASE FOR COMMUNITY-BASED RESTORATION INITIATIVES: AN EXAMPLE FROM HAMPTON ROADS, VIRGINIA. R. D. Brumbaugh, L. Sorabella, C. Oliveras, W. J. Goldsborough, Chesapeake Bay Foundation, 162 Prince George Street, Annapolis, MD 21401. J. Wesson, Virginia Marine Resources Commission, 2600 Washington Avenue, Newport News, VA 23607.

Oysters remain at historically low levels throughout the Chesapeake Bay. Efforts to restore oysters in recent years have focused on establishing a series of sanctuaries, or no-take zones, to increase broodstock populations in selected tributaries. While parasites continue to affect the rate of recovery in these tributaries, innovative management strategies, advances in aquaculture technology, and the availability of parasite-tolerant broodstock from the lower Chesapeake Bay have provided ways to directly involve the public in restoration of this public resource. In 1996, citizen volunteers helped transplant approximately 750,000 large wild-caught oysters onto a one-acre broodstock sanctuary reef in the Great Wicomico River, Virginia. Spawning by those oysters resulted in a 10 to 200-fold increase in juvenile oyster abundance throughout that river in 1997. Using this experience as leverage for involving the public, citizens groups and school programs have been enlisted to grow large numbers of hatchery produced native oysters for restocking similar sanctuary reefs throughout the Chesapeake Bay. These volunteers are now actively engaged in a unique partnership with government management agencies in an effort to restore native oysters to the Chesapeake Bay. Efforts to supplemental natural oyster populations in Virginia using hatchery-produced oysters began in May 1998 with a transplanting of 65,000 hatchery-produced oysters onto two reconstructed, strategically located, sanctuary reefs in Hampton Roads, Virginia. Additional oysters produced by citizen oyster growers will be transplanted in late summer and early fall. The results of citizen's restoration activities to date culminating in this first year's transplanting efforts will be presented.

THE USE OF AN HYDRO-ACCOUSTIC PROCESSING SYSTEM FOR SHELLFISHERIES MAPPING AND RESOURCE MANAGEMENT. G. M. Burnell, Aquaculture Development Centre, University College Cork, Ireland, E. O'Leary and N. Connolly, Coastal Resources Centre, University College Cork, Co. Cork, Ireland, O. Oskarsson, Marin Mattek AB, Box 4082, Vastra Frolunda, Sweden, J. Breslin, Marine Institute, Fisheries Research Centre, Abbottstown, Dublin 15, Ireland, D. Burns, Seabed Surveys International Ltd., The Marina Crosshaven, Co. Cork, Ireland.

A seabed classification system with the trade name RoxAnn™ has been successfully used in resource delineation for several commercial bivalves. It is essentially a "black box" attached to a standard single beam echosounder transducer mounted on the hull of a small vessel. The reflected signals are analysed to give "roughness" and "hardness" coordinates which are displayed in real time as a colour coded track. Once the data has been ground truthed using conventional sampling techniques it is possible to survey at speeds of up to 20 km per hour. The RoxAnn™ track data can then be analysed using MICROPLOT post processing software to produce 3-D maps of the seabed sediments incorporating bathymetric data. RoxAnn™ goes further than seabed mapping as it can detect assemblages of benthic plants and animals which are dense enough to modify the echosounder signals. For example it has successfully detected aggregations of Iceland scallops *Chlamys islandica* and the clam *Mercenaria mercenaria* in Canada and mapped the biomass of mussel beds (*Mytilus edulis*) in Sweden and Ireland. By using a hydrographic 7°, 200 khz transducer it has been possible to reduce the "footprint" down to a diameter of 2.5m at 20m depth and to experimentally detect the scallop *Pecten maximus* in Norway at densities as low as 2 per m². The relevance of this technology to shell fisheries management arises from its ability to accurately map substrates and to locate certain aggregations of shellfish like mussel seed beds. Therefore animals can be matched to habitat and restoration progress can be monitored with regular RoxAnn™ surveys.

DNA-BASED MOLECULAR DIAGNOSTICS FOR *HAPLOSPORIDIUM NELSONI* (MSX) LIFE CYCLE STUDIES. E. M. Burrenson, N. A. Stokes, and B. S. Flores, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062. S. E. Ford, K. A. Alcox, and J. R. Pharo, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

The oyster pathogen *Haplosporidium nelsoni*, the agent of MSX disease, has caused extensive oyster mortality in the eastern United States since 1957. The life cycle stage infective to oysters and the source of that stage have not yet been identified. Attempts to infect oysters directly with *H. nelsoni* spores have been unsuccessful, thus leading to speculation that parasite transmission be-

tween oysters occurs via an obligate intermediate host. We have developed *H. nelsoni* spores that have been unsuccessful, thus leading to speculation that parasite transmission between oysters occurs via an obligate intermediate host. We have developed *H. nelsoni*-specific polymerase chain reaction (PCR) and *in situ* hybridization (ISH) diagnostic assays. These assays have been optimized for use with environmental samples and are being used in the search for the putative intermediate host(s). Samples of water and sediment fractions and of macroinvertebrates have been taken from MSX-endemic areas of York River, VA and Delaware Bay since March 1996. Total genomic DNA has been extracted from each sample and subjected to PCR amplification. Samples that have yielded *H. nelsoni* PCR product have been more frequent from the York River than from the Delaware Bay, corresponding to the MSX disease prevalence in oysters from these locations. ISH is being used to screen PCR-positive macroinvertebrate samples to discriminate between true infections and those where *H. nelsoni* is simply adhering to the external surface or passing through the gut.

THE INFLUENCE OF ECOLOGICAL PROCESSES ON TRANSMISSION OF *PERKINSUS MARINUS*: DISPERSAL, DILUTION, AND HOST FILTRATION. D. Bushek, and A. J. Erskine, Baruch Marine Field Laboratory, Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, PO Box 1630, Georgetown, SC 29442, R. Dame, Department of Marine Science, Coastal Carolina University, PO Box 1954, Conway, SC 29528, Loren Coen and Nancy Hadley, Marine Resources Research Institute, South Carolina Department of Natural Resources, PO Box 12559, Charleston, SC 29442-2559.

Perkinsus marinus is a serious impediment to the recovery of eastern oyster populations (*Crassostrea virginica*). The mechanisms that drive and maintain epizootics remain unclear. Warm temperatures and high salinity are thought to drive epizootics while cold winters followed by low salinity stall epizootics. Some studies show that transmission decreases with distance from infected oysters while other observations imply that transmissible stages are broadly dispersed. A recent model predicts that heavy oyster recruitment can stall an epizootic by reducing the *per capita* infective dose. Conversely, rapid reductions of oyster populations may concentrate transmissible stages in remaining oysters. We are studying transmission dynamics of *P. marinus* within intertidal creeks in South Carolina. Oyster biomass to creek basin volume was equalized among eight intertidal creeks. Specific pathogen free (SPF) oysters were deployed at 0, 50, 100 and 150 m from the creek mouth in August 1997. Infections were detected at all locations after one month and progressed from a mean of 155 to 6,896 parasites g^{-1} between September and January. Distance from creek mouth did not affect infection onset or progression and differences among creeks were not consistent over time, i.e., all sites behaved similarly. After removing oysters from four creeks, new SPF oys-

ters were deployed in February and again in June 1998. Following the spring deployment all oysters became infected within two weeks, but infections progressed little by May 1998 (17 to 52 parasites g^{-1}). Details of this study will be presented including results from summer 1998 and preliminary results from fall 1998.

SUMMER MORTALITY IN PACIFIC OYSTERS: OBSERVATIONS ON THE INFLUENCES OF WATER QUALITY AND CULTURE METHODS. D. P. Cheney, R. Elston, and B. MacDonald, Pacific Shellfish Institute, 120 State Avenue N.E. #142, Olympia, WA 98501-0600 and PO Box 687, Carlsborg, WA 98324.

Pacific oysters (*Crassostrea gigas*) account for the bulk of the oysters produced on the west coast of North America. Virtually, all of these oysters originated from seed stocks imported from Japan between the 1920's and 1970's. Culture of the Pacific oysters began in a small number of rural sites and spread rapidly so that today commercial culture is carried out in many non-urban embayments in Washington, Oregon, northern California, northern Mexico, southern British Columbia and portions of Alaska. While oyster production on the U.S. west coast has not experienced the catastrophic losses from disease plaguing the east coast, mass mortalities periodically occur on commercial farms. Oysters from growing areas in Washington, Oregon and California sporadically experience larger than expected die offs in summer months. Sharp increases in mortality from Puget Sound, Washington from June to September are considered the classic example of west coast summer mortality. More recently, losses of seed oysters in Europe have also been attributed to summer mortality. Work began in early 1998 on a Oyster Disease Research Program study to more precisely characterize summer mortality in a variety of culture conditions and locations, definitively describe the relationship to infectious diseases, and identify water quality and seasonal patterns. Additionally, both a field component that investigates oyster thermal stress response and an assessment of induced thermal tolerance to reduce oyster mortalities are underway. Management practices for commercial cultivation are also being evaluated as possible measures to reduce the frequency and extent of oyster losses.

INTERTIDAL OYSTER REEF HABITAT DEVELOPMENT AND FUNCTIONING: COMPARISON OF DEVELOPED AND REFERENCE SITES AFTER FOUR YEARS. L. D. Coen, E. L. Wenner, D. M. Knott, B. W. Stender, N. H. Hadley, M. Y. Bobo, D. L. Richardson, Marine Resources Research Institute, SCDNR, Charleston, SC 29412, R. E. Giotta, University of Charleston, Graduate Program in Marine Biology, 217 Fort Johnson Rd, Charleston, SC 29412.

The goal of a 1995 meeting in Williamsburg was to review and synthesize current knowledge and approaches concerning oyster reef habitats. There, several projects were nearing completion.

others were just beginning, but most were subtidal with minimal tides. In SC four years ago, we began a long-term study to evaluate intertidal oyster reef development and function. Using an experimental approach, we constructed replicate experimental reefs pairing these with natural reefs at two sites (Inlet Creek-reference and Toler's Cove-developed) differing in urban development and oyster harvesting status. Descriptive and experimental data were collected, including: (1) water quality; (2) associated transient and resident faunas; (3) oyster population studies; and (4) predator-prey (see poster) and Dermo and MSX disease relationships. First, the intertidal physical regime is very different from the subtidal one, especially with respect to temperature. Second, after four years, we have begun to analyze associated transients and residents. Preliminarily, within-site resident species richness was similar between natural and experimental reefs after one year. Faunal densities were similar between reef types at Toler's Cove. Inlet natural reefs supported greater resident densities throughout most of the first year. Biomass showed a different pattern, reflecting *Geukensia* abundance at Inlet. The transient reef community showed no significant differences in overall abundance or species richness between experimental and natural reefs at either site. *Palaemonetes* spp. were a major component on all reefs sampled, often exceeding 150/m². Third for SPF-oysters, subtidal>intertidal growth, but survival was poor. Disease levels were higher at Toler's than Inlet Creek. We are currently sampling transient faunas in tidal creeks of adjacent intertidal oyster reefs, salt marsh and mud flats and examining food web relationships evaluating habitat linkages.

"...ONE BRIEF SHINING MOMENT KNOWN AS CLAMALOT...": THE CEDAR KEY STORY. S. Colson, Suwannee River Water Management District, PO Box 376, Cedar Key, FL 32625, and L. N. Sturmer, University of Florida Cooperative Extension Service, PO Box 89, Cedar Key, FL 32625.

The heritage and culture of rural communities along Florida's Big Bend coastline have been intrinsically linked with commercial fisheries for generations. Over the past decade, closures of oyster harvesting grounds and a state-imposed ban on gill nets triggered economic decline and despair in this area. A transition to shellfish aquaculture as an alternative employment opportunity has been facilitated through the recent efforts of federally-funded, job retraining programs. From the placement of the first program graduates onto leases in 1993, the industry now supports over 250 hard clam growout operations on 950 acres of state-owned submerged lands with sales, farm gate value, in 1997 estimated at \$10 million. The promise of prosperity has created a new excitement and common bond among the individuals that make up these communities. With a renewed sense of purpose and cohesion, people are working together to promote their livelihoods, and above all, to protect the coastal waters so critical to the success of these ventures. This revitalization has also spurred a reaction and responsiveness to the

emergent industry by local governments and by state and federal agencies. Citizens of Cedar Key have formed advisory groups to work closely with elected officials and agency representatives in the planning and implementation of a wide range of water quality activities. These include storm water and wastewater treatment, environmental education and water quality monitoring programs. Sustainable hard clam aquaculture operations have proven to be an excellent opportunity to both protect and preserve the region's environmental qualities as well as support economic activity.

OLYMPIA OYSTER STOCK REBUILDING STRATEGY FOR WASHINGTON STATE. A. E. Cook, Washington Department of Fish and Wildlife, Point Whitney Shellfish Lab, 1000 Point Whitney Road, Brinnon, WA 98320, J. A. Shaffer, Washington Department of Fish and Wildlife, Point Whitney Shellfish Lab, 1000 Point Whitney Road, Brinnon, WA 98320, B. Dumbauld and B. Kauffman Washington Department of Fish and Wildlife, Nacotta Field Laboratory, PO Box 190, Ocean Park, WA 98640.

The Olympia oyster (*Ostrea lurida*) is native to the state of Washington. Once the basis for a thriving, state wide oyster industry, its numbers were drastically reduced by the mid 1940's. Water quality and overharvesting are thought to be the major factors in its near demise. The Pacific oyster (*Crassostrea gigas*) has since replaced the Olympia oyster in Washington and world markets. The Washington Department of Fish and Wildlife is now developing an Olympia oyster stock rebuilding strategy. The goal of the strategy, to restore the Olympia oyster within its historical geographic range, is quite simple, but offers many challenges. Key elements of the draft strategy include historical and current distribution of, habitat and water quality impacts to, interspecies interactions with, and Tribal co-management of the Olympia oyster. Partnering with local commercial shellfish interests and the general public provides new opportunities for restoring the native oyster, and is a top priority for state management of this species. Suggested priorities for implementation of the strategy will be discussed.

SHOREBIRD USAGE AND PREDATION ON OYSTER REEFS AT FISHERMAN'S ISLAND VIRGINIA, U.S.A. J. A. Crockett, M. W. Luckenbach and F. X. O'Beirn, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Wachapreague, VA 23480.

Characterizing ecological function is an essential feature of restoration research. For oyster reef habitats, this research has generally focussed on subtidal processes involving invertebrates and finfish, often overlooking the utilization of the reef by terrestrial and avian predators. Intertidal reefs, however, may provide important habitat and foraging grounds for shorebirds. We conducted a shorebird survey within and adjacent to an area encom-

passing thirteen reefs constructed of three substrates (surf clam shell, oyster shell and coal ash pellets) near Fisherman's Island, VA, U.S.A. All birds found on the reefs and adjacent habitats were identified and counted. We documented the roosting and foraging behavior of the American Oystercatcher *Haematopus palliatus* which feed most extensively on the reefs constructed with oyster shell. We estimated the predation pressure by the American Oystercatcher on oysters by documenting prey capture rates and then extrapolating over the period of aerial exposure. Examination of the reef surface provided an estimate of the size frequency of live oysters and those showing evidence of oystercatcher predation. Our findings indicate that avian predators may have a significant impact on the development of restored oysters reefs and contribute to energy flow from the reef system.

TESTING THE ROLE OF OYSTER REEFS IN THE STRUCTURE AND FUNCTION OF TIDAL CREEKS WITH A REPLICATED ECOSYSTEM SCALE EXPERIMENT: SYSTEM LEVEL VARIABILITY AND RESPONSE TO REMOVAL OF OYSTERS. R. F. Dame, E. Koepfler, L. Gregory, and T. Prins, Coastal Carolina University, Conway, SC 29526, and D. Allen, D. Bushek, C. Corbett, D. Edwards, B. Kjerfve, A. Lewitus, J. Schubauer-Berigan, S. Thomas and students, Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29442.

Data from an ongoing replicated ecosystem level experiment that addresses the ecological role of oyster reefs in tidal creeks is summarized. Geomorphology and hypsometry were determined for eight similar tidal creek systems in North Inlet Estuary, South Carolina, U.S.A. Oyster biomass, which ranged from 2 to 24 g dry wt. m^{-3} of water, was standardized to 8 g dry weight m^{-3} . Subsequently, several structural and functional attributes (water quality, phytoplankton and bacterial productivity, oyster growth and recruitment, nekton utilization, total creek metabolism and nutrient cycling) were monitored in each creek for one year to determine system variability. In January 1998, after the first year of monitoring was complete, oyster reefs were removed from four of the eight creeks in a randomized block design. Monitoring continued so that Before reef removal and After reef removal data can be compared among Control (no reefs removed) and Impact (reefs removed) creeks in completely replicated BACI design. Pre-reef removal data indicated high seasonal variability and significant variability among creeks. Relative differences among creeks were stable—creeks generally maintained the same rankings throughout the year. Analysis of subsequent monitoring will look for changes in the behavior of creek attributes before and after oyster reef removal. This study will span El Niño and La Niña events as well as intervening years. The BACI design accounts for such overriding effects, enabling this study to not only examine the impacts of removing oysters, but also examine differences in system response to major perturbations when oyster reefs are present or absent.

SHELLFISH STOCK ENHANCEMENT, MANAGEMENT AND RESTORATION IN THE BAY OF MONT SAINT-MICHEL, BRITTANY FRANCE. J. C. Dao, IFREMER Centre de Brest, 29280 Plouzane, France.

The Bay of Mont Saint-Michel is located on the west part of the English channel off France. It belongs to a larger coastal ecosystem called "Normand-Breton Gulf" (ICES area VIIe) of 8400 Km² open to the west and the north, including the channel islands (United Kingdom). The large and shallow area is remarkable for the large amplitude of the tides and for the size and diversity of the bivalves communities. Shellfish exploitation is based on farming (flat-oyster, cupped oyster, mussel) and part of the fishing in the gulf (native flat oyster, warty-venus, clams, pectinids). Production in the bay averages 7000 tonnes of oysters and 10000 tonnes of mussels annually. Most of the shellfish farming requires the introduction of juveniles since there is no natural recruitment. Management is based on the primary productivity on intertidal areas devoted to a single species and delimited by private leases. Professional regulations (biomass and spatial distribution) are regularly reviewed by the shellfish farmers organizations. Interactions with the open ecosystem (invasion by a competitive gastropod, *Crepidula fornicata*), and water quality in the estuarine areas are taken in account.

ENTEROCOCCI AS A TRACER OF WASTE DISCHARGE CONTAMINANTS IN ESTUARINE SHELLFISH. S. De Luca, G. D. Lewis, and R. G. Creese, University of Auckland, School of Environmental and Marine Science, Leigh Marine Laboratory, PO Box 349, Warkworth, New Zealand.

Wastes are often discharged into estuaries and harbours, and commonly contain contaminants such as heavy metals, pesticides, detergents, and synthetic chemicals which are likely to adversely effect human health and marine organisms. Faecal bacteria, also found in such waste discharges, can be used as quick, simple and inexpensive tracers of these contaminants. Faecal bacteria are accumulated by shellfish in a similar way to contaminants, and their presence in water, sediment and shellfish tissue samples can indicate the potential presence of other pollutants. *Enterococci* were used to trace storm water and other discharges in a NE New Zealand harbour where *Austrovenus stutchburyi* (little neck clam) and *Macomona liliana* (wedge shell) are commonly found. Shellfish tissue samples taken 2-monthly over 2 years indicate a seasonal nature to the levels of bacteria detected. High levels in winter correlate with increased rainfall and, therefore, greater runoff and stormwater flows. A further experiment was carried out over 12 days in winter to ascertain the effect of rainfall events on levels of *Enterococci* in water, sediment and shellfish tissue. The results indicated an immediate increase in levels of bacteria in stormwater with high rainfall. Harbour water, clam tissue and sediment were similarly affected the day following high rainfall, but tissue from the deposit-feeding wedge shell was not significantly affected.

Shellfish and human health are likely to be at greater risk from lethal and sublethal effects of contaminants contained in waste discharges during winter months or episodes of high rainfall, when runoff and stormwater flows are greatest.

INDICATORS OF EFFECTS OF WASTE DISCHARGES ON THE NEW ZEALAND LITTLE NECK CLAM *AUSTRO-VENUS STUTCHBURYI*. S. D. De Luca, R. G. Creese, and G. D. Lewis, University of Auckland, School of Environmental and Marine Science, Leigh Marine Laboratory, PO Box 349, Warkworth, New Zealand.

Coastal waters are commonly used for disposal of sewage, stormwater and other wastes, and it is these same coastal areas that are used for recreation and shellfish gathering. This research is to determine robust indicators of shellfish "health" which are sensitive to waste inputs such as stormwater, sewage and septic tank leachate. A multidisciplinary approach is used, with a broad array of microbiological, ecological and biochemical parameters being investigated. Experiments using enterococci as a tracer of these discharges indicated that during high rainfall the concentration of enterococci significantly increased in stormwater point source, harbour water, surficial sediment and clam flesh. Another site, which has several stormwater drains and streams discharging into it and where there are houses (and therefore septic tank leach fields) close to the foreshore, was chosen as the focus of a study testing potential biochemical indicators of metabolic stress. A reference site with no houses nearby or point source inputs was also studied. The biochemical methods used for the determination of glycogen, adenylate energy charge and total adenylate pool in clam foot muscle are presented. Preliminary results indicate no significant difference between sites for glycogen or adenylate energy charge. However, total adenylate pool was higher at the reference site than the putative impact site. As a consequence of these results, controlled experiments in running seawater aquaria are currently being carried out in order to ascertain at what concentration of specific contaminants the biochemical indicators are affected in *A. stutchburyi*.

THE BRAS D'OR LAKES OYSTER INDUSTRY. C. Dennis, Eskasoni Fish and Wildlife Commission, Eskasoni, Cape Breton, N.S. BOA 1JO, Canada.

In times past, the River Denys Basin has supported a thriving wild oyster industry. Aquaculture for oysters increased during the 1970's and 80's. Due to changes in the market, the industry dried up. There has recently been a rebirth in interest in the aquaculture industries all over the Atlantic provinces. Eskasoni First Nation has been attempting to restore the oyster aquaculture industry through the presentation of a workshop in Orangedale which discussed the problems faced by the industry, including the lack of reliable seed, the closing of shellfish areas through sewage con-

tamination and the lack of reliable markets. Through the workshop, the Eskasoni Fish and Wildlife Commission agreed to begin two tasks—to help implement a stakeholders group which will address the problem of sewage contamination in the basin and to implement an Oyster Growers Association which could act as an advocate for oyster aquaculture in the Lakes. There has been interest expressed by various government agencies to assist in the development of these groups to bring the oyster industry back to its former prominence in Nova Scotia.

MAINTAINING THE SHELLFISH NICHE IN THE NEW MILLENNIUM—THE PACIFIC COAST OYSTER GROWERS ASSOCIATION APPROACH. W. F. Dewey, Taylor Shellfish Company, 130 S.E. Lynch Road, Shelton, WA 98584.

Intense population growth, the associated pollution, coupled with demand for competing uses of our marine estuaries (views by NIMBY's, recreation) are jeopardizing the survival of West Coast shellfish farmers. Watershed ecosystem management has become the rage as resource managers and politicians grapple with balancing the demands and impacts of this increased growth. The Pacific Coast Oyster Growers Association recognizing if they are to maintain shellfish's niche in that watershed ecosystem, growers need to be proactive on several fronts. Shellfish farmers need to walk the talk. No longer is it adequate to go to hearings, profess themselves as the canaries in the mineshaft and ask everyone upstream to fix their problems. PCOGA is embarking on an effort to develop coast wide shellfish farming Best Management Practices (BMP's). They are exploring developing those BMP's into a programmatic Habitat Conservation Plan to provide regulatory stability under the Endangered Species Act and impending salmonid listing. The Pacific Shellfish Institute (PSI), the research arm of PCOGA is pursuing funding for shellfish ecosystem research as its highest priority. PCOGA members are being encouraged to get involved on local watershed planning committees, with growth management planning, on Conservation Districts, with environmental groups and other watershed stakeholder groups to educate as to the value of a healthy shellfish resource as part of the ecosystem. Only armed with this proactive arsenal and establishing ourselves as the most responsible user group in the ecosystem will shellfish farming survive into the new millennium.

BLUE MUSSEL FISHERY IN DENMARK. P. Dolmer, Danish Institute for Fisheries Research, Dep. of Marine Fisheries, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark.

The blue mussel, *Mytilus edulis*, is a dominant species in Danish coastal waters. The species forms dense mussel beds and is an important controlling component of pelagic primary producers. The mussel stocks are exploited by an extensive fishery resulting in comprehensive reductions of local mussel populations. Danish Institute for Fisheries Research (DIFRES) has for years partici-

pated in studies on the direct effect of the fishery of mussels by dredge such as resuspension of sediment and disturbance of benthic invertebrates. In addition, studies on mussel population dynamics, mussel bed structures and the ecological role of the mussels enable an evaluation of the indirect effects of mussel dredging. In 1996 the effects of mussel dredging on the benthic community was studied. A diver identified commercial dredging tracks, and the benthic infauna was sampled by van Veen grab inside the tracks and just outside. A field experiment analysed the effect of dredging at the benthic fauna in four study sites. At two of the plots, experimental dredging exposed half of the plot. The fauna at the study sites was quantified by sampling before dredging, and again one day, one week, and one month after dredging. The infauna was sampled by van Veen grab and the epifauna was quantified from UW-photos. The talk integrates the results from the different studies in order to evaluate the mussel fishery in a larger ecosystem context.

AN ECONOMIC ANALYSIS OF AN OYSTER HABITAT RESTORATION PROJECT TO OFFSET DAMAGES DONE BY HURRICANE ANDREW TO THE OYSTER RESOURCES IN CENTRAL, COASTAL LOUISIANA. **R. J. Dugas**, Louisiana Department of Wildlife and Fisheries, 1600 Canal Street, New Orleans, LA 70890, **W. R. Keithly**, Center for Coastal Energy and Environmental Resources, Louisiana State University, Baton Rouge, LA 70803, **D. Lavergne**, Louisiana Department of Wildlife and Fisheries, PO Box 98000, Baton Rouge, LA 70898.

On August 25, 1992, Hurricane Andrew devastated the Louisiana coast east of Atchafalaya Bay, passing through several of the designated public oyster grounds. Sustained winds near the center of this Category 4 storm were 130 mph for five hours or longer, and caused Gulf water surges in excess of 20 feet at the shoreline. These surges displaced substantial amounts of marsh and bottom sediments redepositing them throughout the adjacent waterbottoms in the area. Some of the state's most productive oyster reefs were in these waterbottoms that were buried by the displaced sediment and accompanying vegetation. The immediate assessed value of the damage to the impacted area, which did not include the long-term impact of the lost habitat necessary for future production, was estimated in excess of \$26 million. Under the Interjurisdictional Fisheries Act Program, administered by the U.S. Department of Commerce's National Marine Fisheries Service, Louisiana received \$5.1 million federal funding for restoration efforts of the oyster communities on some of the Louisiana public oyster grounds. This presentation will concentrate on the economic returns from this effort to restore damaged oyster resources in the Terrebonne Parish region, which was in the direct path of Hurricane Andrew. Monitoring oyster harvesting activities since the restoration efforts have indicated a significant benefit to cost ratio.

USE OF OYSTER SHELL TO CREATE HABITAT FOR JUVENILE DUNGENESS CRAB IN WASHINGTON COASTAL ESTUARIES: STATUS AND PROSPECTS. **B. R. Dumbauld** and **B. E. Kauffman**, Washington Department of Fish and Wildlife, Willapa Bay Field Station, PO Box 190, Ocean Park, WA 98640, **D. A. Armstrong** and **E. Visser**, School of Fisheries, Box 357980, University of Washington, Seattle, WA 98195.

The deployment of oyster shell in estuarine intertidal areas to create habitat for juvenile Dungeness crab (*Cancer magister*) is now routinely used as a mitigation technique for "unavoidable losses" of crab during dredging operations in Grays Harbor and Willapa Bay along the southwest coast of Washington State. Original feasibility studies were conducted in 1986/87 for a project administered by the U.S. Army Corps of Engineers which widened and deepened the navigation channel in Grays Harbor. Since that time, several studies have elucidated the ecology of crab and other organisms that recruit to the shell reefs that are created and have also refined the procedures used to calculate the number of crabs lost due to dredging and those produced by the shell placement. While the shell produces crab habitat, initial assumptions about the longevity of the shell have proved to be overly optimistic and the shell often sinks or is covered with silt before the end of the first summer after deployment. In addition, competition with shore crabs *Hemigrapsus oregonensis*, has caused juvenile Dungeness crab to be displaced. We summarize results of these studies, make some ecological comparisons of habitat value with "natural" habitats such as eelgrass that also act as intertidal crab nursery areas, and present initial results from an ongoing mitigation effort which seeks to produce a more persistent living oyster reef in Willapa Bay.

POTENTIAL USE OF RAY'S FLUID THIOGLYCOLLATE MEDIUM TO DETECT AND QUANTIFY PERKINSUS MARINUS IN ENVIRONMENTAL WATER SAMPLES. **R. C. Ellin** and **D. Bushek**, Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, PO Box 1630, Georgetown, SC 29442.

Perkinsus marinus is a major pathogen of the eastern oyster, *Crassostrea virginica*. Many studies have examined the physiological responses of *C. virginica* to *P. marinus* and host-parasite responses to changing physical parameters (salinity and temperature); however, few studies have investigated the transmission dynamics that drive this relationship. Horizontal transmission of *P. marinus* occurs via the water column. Accurate methods for quantification of *P. marinus* in the water column are necessary to study transmission dynamics. *P. marinus* in environmental water samples can be enumerated with competitive molecular PCR assays or an antibody probe coupled with flow cytometry. These methods are expensive and there is some uncertainty surrounding possible cross reactivity of the antibody probe. Ray's Fluid Thioglycollate Medium (RFTM) assay is an inexpensive, accurate

methodology to assess *P. marinus* infection intensities within oyster tissue, hemolymph, feces, and pseudofeces. Documentation of the use of RFTM to analyze environmental water samples does not exist. We used RFTM to detect *P. marinus* in environmental water samples. Experiments investigating the feasibility of the use of RFTM as a method of *P. marinus* enumeration in environmental water samples will be presented.

SHELLFISH HEALTH MANAGEMENT FOR ENHANCEMENT, RESTORATION AND CULTURE ON THE WEST COAST. R. A. Elston and D. Cheney, Pacific Shellfish Institute, PO Box 687, Carlsborg, WA 98324 and 120 State Avenue N.E. #142, Olympia, WA 98501-0600.

Pacific and kumamoto oysters (*Crassostrea gigas* and *C. sikamea*) and manila clams (*T. philippinarum*) are widely cultured on the west coast of North America. Native bivalve culture is relatively limited by several species such as the geoduck clam (*Panope abrupta*) and native littleneck clam (*Protothaca staminea*) are part of an emerging technology that supplies seed and adult shellfish for enhancement, restoration, and commercial production. It is essential that health management procedures be in place to prevent the dissemination of infectious diseases during these activities and to reduce operational costs. Studies on cultured seed stock in Washington, Oregon, California and Hawaii revealed opportunistic but significant bacterial pathogens and several other conditions. No diseases considered certifiable were found. A broader mortality and health study of adult oyster stocks is underway to elucidate causes of periodic mortality and establish an ongoing program of health surveillance. Developing technology for new species in culture must include health management and disease prevention in the intensive culture facilities. Morphologic pathology is the basis of disease evaluation but needs to be supplemented by microbiological and molecular methods for diagnosis and characterization of infectious diseases. Shellfish health programs for commercial producers are under development in order to better understand the disease process related to shellfish mortalities and product losses, and to foster practices that will enhance shellfish health. The health program consists of standards for brood stock management, hatchery and grow out operations, a response plan for disease outbreaks, record-keeping requirements and standards for evaluation and training.

INVESTIGATION OF THE STRESS RESPONSE, SUMMER MORTALITY AND DISEASE RESISTANCE OF OYSTERS, *CRASSOSTREA GIGAS* AND *CRASSOSTREA VIRGINICA*. C. S. Friedman, G. N. Cherr, J. S. Clegg, A. H. Hamdoun, J. L. Jacobsen, S. A. Jackson, and K. R. Uhlinger, Bodega Marine Laboratory, Univ. California Davis, PO Box 247, Bodega Bay, CA 94923.

The ability to mount a stress response is often essential for an organism's survival, especially for oysters inhabiting dynamic and stressful environments which experience summer mortalities. We

have characterized and response of Pacific oysters, *Crassostrea gigas*, to heat shock, hypo-osmotic acclimation and disease. Pacific oysters synthesized heat shock proteins (HSP) in the 70 kD family and exhibited prolonged induced thermotolerance (ITT, at least 3 wk) after exposure to heat shock. Hypo-osmotic acclimation delayed HSP production and tolerance to lethal temperatures. Oysters challenged with *Nocardia* synthesized HSPs in a pattern similar to control animals. However, the degree of ITT was reduced in oysters with nocardiosis. We are investigating the interaction between heat shock, nocardiosis and immune function in order to assess the role of several stressors and host defense/repair mechanisms in summer mortality. Oysters held at 12°C required an elevation in temperature of over 21°C to induce HSP production. Animals acclimated to 22°C produced HSPs after a temperature increase of only 15°C (heat shocked at 37°C). Thus, the temperature needed to induce a stress response may be related to the upper thermal limit of the oyster (43.5–44°C) and may not be based solely on the magnitude of thermal shock. The Eastern oyster, *C. virginica*, produced HSPs after heat shock in the same manner as the Pacific oyster. However, the magnitude of ITT was reduced relative to that of Pacific oysters held under the same culture and experimental conditions.

COOPERATIVE REGIONAL OYSTER BREEDING PROJECT. S. K. Allen, Jr., and P. M. Gaffney, Aquaculture Genetics and Breeding Center, Virginia Institute of Marine Science, Gloucester Point, VA 23062 and College of Marine Studies, University of Delaware, Lewes, DE 19958.

CrosBreed is a cooperative regional program involving investigators from Delaware, Maryland, New Jersey, and Virginia, states whose oyster industries have been hard hit by the oyster diseases MSX and Dermo. Its primary goal is to evaluate the performance (growth and survival in the presence of both MSX and Dermo) of several selected oyster lines in several mid-Atlantic environments. Five oyster lines created in 1995 from first generation Delaware Bay synthetic lines selected for resistance to MSX were deployed at three sites (VA, MD and NJ) in August 1995. A locally produced control group as well as a global control group (of New Jersey origin) were also deployed at each site. At regular intervals from fall 1995 through fall 1997, samples from all groups at all sites were examined for MSX and Dermo, as well as overall growth and survival. In the two higher salinity sites (VA, NJ) both growth and survival were markedly better in CROSBreed lines than control lines, with all groups showing high survival during the first 12–18 months. At the lower salinity site (MD), survival was poor during the first year but high afterwards, with the local control performing best. No differences in growth rates among CROSBreed and control groups appeared at this site. All lines developed Dermo at the VA and NJ sites. CROSBreed lines showed lower disease frequency and prevalence than the global controls at the VA site and performed comparably at the NJ site. Little or no

Dermo appeared at the MD site. Little MSX was observed at any site. Even in the absence of strong MSX pressure, the CROSBreed lines performed well, demonstrating their current value as well as their potential for further development of dual-disease resistant lines. These results also demonstrate the value of controlled field trials in evaluating the performance of selected lines.

PHYSIOLOGICAL AND GENETIC CORRELATES OF Dermo DISEASE RESISTANCE IN THE EASTERN OYSTER. M. Faisal, S. Kaattari, and P. M. Gaffney, Virginia Institute of Marine Science, Gloucester Point, VA 23062 and College of Marine Studies, University of Delaware, Lewes, DE 19958.

We are using a set of 14 full-sib families, which represent genotypes from nearly the entire geographical range of *C. virginica*, to explore genetic and physiological sources of variation in host resistance to Dermo disease. Objectives of our project are: 1) to determine the correlation between putative physiological indicators of disease resistance and actual resistance as measured by natural disease challenge; 2) to determine the genetic component of variation in the putative physiological indicators of disease resistance; 3) to determine the genetic component of variation in Dermo disease resistance after natural disease exposure; 4) to develop additional nuclear DNA markers for the eastern oyster genome map; 5) to determine inheritance and linkage relationships among new and existing genetic markers (DNA and protein) using these pedigreed reference families. Variation among families will provide a first estimate of the genetic component of disease resistance and the genetic contribution to variation in the measured physiological traits. Correlations between measured physiological traits and disease resistance will be evaluated to determine whether the former can serve as good indirect estimators of the latter. In addition to further clarifying the role of these physiological processes in disease resistance, this information may provide ODRP-sponsored efforts to develop a reference set of pedigreed families for genomic mapping of the oyster, and represents a first step toward mapping of markers associated with disease resistance genes in oysters.

WHERE ARE THE SUBTIDAL OYSTERS? AN EXPERIMENTAL EVALUATION OF PREDATION, TIDAL ELEVATION, AND SILTATION ON OYSTER (*CRASSOSTREA VIRGINICA*) DISTRIBUTION IN SOUTH CAROLINA. R. E. Giotta, Grice Marine Biological Laboratory, 205 Fort Johnson Road, Charleston, SC 29412, L. D. Coen, Marine Resources Research Institute, SCDNR, 217 Fort Johnson Road, Charleston, SC 29412.

Oyster (*Crassostrea virginica*) populations in the southeastern U.S. are primarily intertidal, making them distinct from the well studied subtidal populations of the northeastern U.S. and the Gulf of Mexico. Four concurrent experiments examined how physical

and biological factors interact to affect oyster distribution in a South Carolina tidal creek. Three tidal positions (intertidal, subtidal on-bottom, and subtidal off-bottom) and two predator treatments (exclusion cage and partial cage, or control) were employed to evaluate effects of tidal elevation, predation, and siltation on survival and growth of hatchery-reared oysters and on long term (7 mos.) oyster recruitment. Preliminary results for small (1–2 mm SH) oysters suggest that predator exclusion and tidal position did not have a significant effect on oyster survival. For larger (26–40 mm SH) oysters, however, survival was significantly greater in exclusion cages than controls at the two subtidal position; there was no significant difference between caged and control survival intertidally. Oyster growth was not affected by exclusion cage, but was higher subtidally on- and off-bottom than intertidally. Long term oyster recruitment varied significantly with tidal position. More oysters with a smaller final mean size recruited to the intertidal trays than to the subtidal, on-bottom trays; recruitment to the subtidal off-bottom trays was not different from the other two positions. Preliminary results regarding effects of siltation appear equivocal and are discussed. In summary, results indicated that subtidal oyster growth exceeds intertidal growth, but without protection from predation survival is low subtidally. Predation appears to be an important factor limiting subtidal oysters in this area.

WATERMEN, RESEARCHERS, AND COMMERCIAL AQUACULTURISTS WORKING TOGETHER TO RESTORE MARYLAND'S EASTERN AMERICAN OYSTER POPULATION. M. Gluis, South Australian Research and Development Institute (SARDI), South Australia.

The Chesapeake Bay population of the Eastern American Oyster (*Crassostrea virginica*) has been substantially reduced by the effects of the pathogenic organisms *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX). This has had a large environmental, social and economic impact on the Chesapeake Bay and watermen that derive income from the collection of these oysters. This project was conducted jointly with the Maryland DNR, Corp of Engineers and the Maryland Watermen Association. The main goal was to produce as many oysters as possible and use these to re-seed areas that were known to have a low incidence of 'Dermo' and 'MSX'. Added benefits to this were: 1) The demonstration of aquaculture methods as an alternative for watermen to enhance viability of their traditional 'wild catch' industry, 2) The restoration of the The Maryland Department of Natural Resources' Piney Point Aquaculture Center to operational standard, 3) The development of microalgae and oyster nursery skills of the Maryland DNR staff, 4) The increased settlement and survival of Eastern Oyster larvae grown using an adequate diet and reduced stocking density, 5) Settlement rates of culched and culchless spat was 19% and 66% respectively. These spat were then transferred to nursery sites within the Chesapeake Bay.

ENHANCING A TRADITION: OYSTER FARMING FOR CHESAPEAKE WATERMEN. **D. L. Leonard**, National Marine Fisheries Service, 1315 East West Highway, Silver Spring, MD 20910, **D. J. Grosse**, Terraqua Environmental Science and Policy, LLC, 3754 Jenifer Street, N.W., Washington, DC 20015, **R. S. Kallen**, RSK Strategies, 1870 North Larabee Street, Chicago, IL 60614, **R. C. Karney**, Martha's Vineyard Shellfish Group, Box 1552, Oak Bluffs, MA 02557.

The 1,200 inhabitants of Smith and Tangier islands are direct descendants of British colonists who settled these islands in the early 1700s. Since the mid-1800s the islands' economies have depended upon crab and oyster harvesting. The recent declines in shellfish resources threaten the livelihood of the local watermen. To offer an additional economic option compatible with the island's unique lifestyle we designed a project which develops an integrated oyster industry, capable of producing, processing and marketing oysters year-round. The goal is to offer watermen an additional economic option while supporting these islands and their unique lifestyle. Project success will depend on the number of watermen involved, their success in raising and marketing oysters and their ability to make the industry economically self-sustaining. While the major diseases that contributed to the oyster decline (dermo and MSX) will likely persist for the foreseeable future, the impact of the disease can be minimized by: (1) brood stock selection for disease tolerance and fast growth; (2) minimizing disease exposure by delaying spawning and using low salinity nursery sites; and (3) using techniques such as off-bottom culture to promote faster growth and minimize disease impacts. Increasing production is only half of the equation. The remaining challenge is getting the oysters to markets. In cooperation with the watermen, we are developing a marketing plan to maximize income and employment by identifying markets, determining costs of producing and delivering oysters to markets, and estimating anticipated revenues from (1) clump oysters and (2) singles or cultchless oysters.

RECYCLING OF NUTRIENTS FROM SEA TO LAND WITH MUSSEL (*MYTILUS EDULIS*) CULTIVATION. **J. Haamer**, Institute of Coastal Research, National Board of Fisheries, Nya Varvet, byggnad 31, 42671 Vastra Frolunda Sweden.

Benthic suspension feeders like mussels have a dominant influence on the flux of nutrients. In the Baltic Sea *Mytilus edulis* colonize the hard bottoms from the surface down to more than 30m depth and in one investigated area on the Swedish coast *Mytilus* amounted to 86% of animal ash free dry weight on the hard bottoms. Owing to a low salinity (0.7% PSU) there is little competition for space and the main predators like crabs and starfishes are absent in the Baltic. The large biomass of mussels in the Baltic is considered to have a stabilizing effect on the ecosystem and decreases the negative effects of eutrophication by removing phytoplankton biomass from the pelagic system. On the Swedish West Coast most sill fjords suffer from temporary anoxia in the deep

basins due to a weak water exchange and eutrophication. In 1997, a project started aiming at an improvement of the oxygen conditions by increasing the mussel populations in the fjords. The increment of mussels is attained by on and off bottom farming for human consumption. The main part of these waters is allowed for shellfish growing as a majority of the sewage systems are connected to well developed purification plants.

OYSTER REEF COMMUNITIES IN THE CHESAPEAKE BAY (ORCCB): A PUBLIC EDUCATION TOOL SUPPORTING OYSTER REEF RESTORATION. **J. M. Harding**, **V. P. Clark**, and **R. Mann**, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Current Chesapeake Bay oyster restoration efforts focus on oyster reefs as living habitat supporting complex ecological communities. Public support of ongoing oyster restoration efforts depends on an awareness of the oyster's commercial importance and the major interconnections between oyster reef communities and water quality, recreational fisheries, and tidal communities. We are developing a research-based public education tool, Oyster Reef Communities in the Chesapeake Bay (ORCCB) that synthesizes information on oyster reef and tidal habitat ecology, scientific methods, and ongoing research projects within the Chesapeake Bay. Connections between tidal habitats, successful recreational fisheries, and oyster reef community health and restoration will be emphasized within a modular framework. The main objective of the ORCCB software is to clearly describe the background and results of our active reef research program into relevant information easily accessible by the general public. The ORCCB CD includes interactive, data-based management scenarios as well as suggestions for classroom resources and activities. This software will be supported by full documentation and an Internet web site.

OYSTER REEF RESTORATION AS A HABITAT ENHANCEMENT TOOL FOR RECREATIONALLY VALUABLE FINFISHES. **J. M. Harding** and **R. Mann**, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

The Chesapeake Bay provides nursery and feeding habitat for many recreationally and commercially valuable finfish species. Three dimensional living oyster reefs provide natural habitat enhancement for fishes such as striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*). Diet and distribution patterns of these fishes across seasonal and diurnal scales were examined at three study sites in the Piankatank River, Virginia. Fish were collected with gill nets from a sand bar, a shell bar, and a three dimensional oyster shell reef during thirty-six hour sampling stations in 1996 and 1997. Bluefish and striped bass were consistently more abundant on the oyster reef than at either of the off reef sites. Teleosts were dominant prey items for both bluefish and striped

bass; fish caught on the oyster reef consumed more species of teleosts than fishes from non-reef sites. Small benthic reef fishes such as naked gobies (*Gobiosoma bosc*) were frequent prey items for reef-associated bluefish and striped bass. In 1996, naked goby and striped blenny (*Chasmodes bosquianus*) abundance on the reef declined in late June during the same time window that striped bass abundance around the reef peaked. Length at age relationships for bluefish and striped bass suggest that reefs are preferred habitat for young (~ Age 1–2) fish of both species. Continued restoration of oyster reefs and the associated communities will enhance habitat for these species and other important finfishes.

MODELING THE MSX PARASITE IN EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) POPULATIONS: MODEL DEVELOPMENT, IMPLEMENTATION AND VERIFICATION. E. E. Hofman and J. M. Klinck, CCPO Crittenton Hall, Old Dominion University, Norfolk, VA 23529, S. Ford and E. N. Powell, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

A model simulating the host-parasite-environmental interactions of Eastern Oysters (*Crassostrea virginica*) and the pathogen, *Haplosporidium nelsoni*, which causes the disease MSX, has been developed. The model is physiologically-based and is structured around proliferation and death rates of *H. nelsoni* under different environmental conditions. Equations describing these rates were constructed using data from long-term field observations, and field and laboratory experiments. Simulations that use environmental conditions characteristic of Delaware Bay reproduce the observed seasonal *H. nelsoni* cycles and consequent oyster mortality. These simulations show the effect of environmental factors, such as salinity and cold temperatures, on controlling the intensity and prevalence of *H. nelsoni* infections. However, biological controls from density-dependent feedback on *H. nelsoni* proliferation and *H. nelsoni* sporulation events also greatly affect disease prevalence and intensity. The oyster-*H. nelsoni* model provides a quantitative framework for guiding future laboratory and field studies as well as management efforts.

MISSISSIPPI SHELLFISH RESTORATION AND ENHANCEMENT PROJECT. J. D. Jewell, S. Gordon, and M. Buchanan, Mississippi Department of Marine Resources, 1141 Bayview Avenue, Suite 101, Biloxi, MS 39530, B. Randall, Gulf Coast Research Laboratory, PO Box 7000, Ocean Springs, MS 39566-7000.

Mississippi experienced extreme adverse weather conditions, including storms, hurricanes (Andrew, Erin, and Opal), and heavy rainfall, from August 1992 through December 1995. These events cause significant flooding, oyster mortality, and degradation of oyster habitat through sedimentation and over burden. Mississippi's oyster reefs are primarily located in the western Mississippi

Sound near the confluence of several major river systems. These rivers crested well above flood stage for extended periods during the disaster period. This major influx of fresh water and sediments caused mortalities on oyster reefs and resulted in heavy infestation of near shore oyster reefs by the hooked mussel, *Ischadium recurvum*. Encrustation by this mussel rendered entire reefs unharvestable. Mississippi Department of Marine Resources (MDMR) personnel assessed damage to the oyster reefs, and submitted an application to the U.S. Department of Commerce (NOAA-NMFS) for funding of the restoration and enhancement efforts which included surveying of reef areas, cultivation of reef areas, and planting of cultch materials. MDMR personnel surveyed reef areas to determine which areas were excessively silted over and assessed which areas needed cultch material or areas that were suitable for oyster reef development. Two modified dredges were towed over the reefs to re-expose existing shell and to break up and reduce the hooked mussel encrustation. MDMR contracted the planting of cultch materials. Cultch materials were transported to pre-selected sites on barges and deployed by high-pressure water hoses, spreading them evenly in a thin layer over the bottom. Preliminary results from these efforts are presented.

POOR WATER QUALITY? NOT IN MY BACKYARD! THE EFFECTIVENESS OF NEIGHBORHOOD POND ASSOCIATIONS IN THE PROTECTION AND IMPROVEMENT OF SHELLFISH GROWING WATERS ON MARTHA'S VINEYARD. R. C. Karney, Martha's Vineyard Shellfish Group, Inc., PO Box 1552, Oak Bluffs, Mass. 02557.

Neighborhood pond associations have proven to be an especially effective force in the protection and enhancement of local coastal ecosystems. Environmental managers who are wise enough to forge partnerships with these local organizations will be rewarded with the energy, commitment, and passion reserved for issues that hit close to home. With the vigilante zeal of a Neighborhood Crime Watch, local pond associations are the eyes and ears that sound the first alerts of environmental pollution. With the efficiency of a local militia, they rally to the cause as volunteers with financial commitment and effective political organization. The environmental accomplishments of neighborhood groups on Martha's Vineyard are impressive. The Edgartown Harbor Association funded a water quality study resulting in the establishment of a free septage pumpout facility for boaters. The Friends of Sengekontacket (Pond) provided the leadership to coordinate a local, state and federal partnership to complete a major dredging project which restored filled shellfish habitat and nourished an eroding barrier beach. The Lagoon Pond Association funded a court battle to limit pier construction. The Tisbury Great Pond Think Tank and Tisbury Water Ways have addressed farm and roadway runoff with fencing, buffer strips and innovative catch basins. All of the associations have conducted successful public education programs, water quality monitoring studies, protective

zoning initiatives and fundraising activities to protect shellfish habitats and water quality.

THE DEMAND FOR OYSTER RELAYING ACTIVITIES IN LOUISIANA, 1973–96. **W. R. Keithly** and **A. Diagne**, Center for Coastal Energy and Environmental Resources, Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803, **R. J. Dugas**, Louisiana Department of Wildlife and Fisheries, 1600 Canal Street, New Orleans, LA 70890.

Approximately one-third of Louisiana's oyster growing waters, much of it privately leased, is classified as either restricted or prohibited at any given time. Relaying of oysters from closed to approved waters is a costly process. As such, the demand for relaying activities is likely to be more prevalent under certain economic and environmental conditions. The demand for relaying can be inferred from the number of permits issued on an annual basis. The demand for these permits, furthermore, is a derived demand and, according to economic theory, should respond positively to increase in the output price of the final product (i.e., the harvested oyster price) and negatively to increases in input costs. Theory would also suggest that the demand responds inversely to relative oyster abundance. Specifically, the greater the overall abundance of oysters the less need there is to incur the additional costs associated with relaying as a means to secure sufficient quantities of the marketable product. To test this theory, the number of permits issued on an annual basis during the 1987–95 period was regressed against the deflated price of the harvested product, an indicator of oyster abundance, and a discrete demand shifter to "capture" the influence of additional costs that were incurred post 1987 associated with the added requirement that a bonded enforcement agent be on site when relaying activities are taking place. The results, in general, conformed to the theory. Specifically, the analysis suggested that a one percent increase in the deflated price of the harvested product resulted in a more than four percent increase in the demand for permits, *ceteris paribus*. Likewise, the increased costs associated with the requirement that a bonded enforcement agent be present resulted in a statistically significant decline in the demand for relaying activities, *ceteris paribus*. The results derived from the analysis will be the basis of the presentation.

VOLUNTEERS CAN DO MORE THAN DIG A DUCK. **T. King**, Washington Sea Grant Program, University of Washington, 11840 N. Hwy 101, Shelton, WA 98584, **H. Beattie**, Washington State Department of Fish and Wildlife, 1000 Point Whitney Road, Brinnon, WA 98320.

Geoduck clams, often called gooeey ducks or G.O. ducks, are perhaps the most talked about clam species by visitors to the Pacific Northwest. The geoduck's native range is from Baja, California to Alaska from the +3 foot tidal height to -400 feet deep,

and lives for over 100 years. The cultivation of geoducks has been underway in Washington State since 1970. With the cultivation of hatchery seed and the development of predator exclusion devices, enhancement of public beaches is a viable venture. Currently, the enhancement of recreational beaches generally yields a 20–40% survival rate. However, the labor needed to install the exclusion devices can often make the enhancement time consuming and expensive. Since 1996, Washington Sea Grant and the Washington Department of Fish and Wildlife have been working together to involve groups such as 4H, Boy Scouts, Girl Scouts and community members to help enhance local recreational beaches. This effort has lead to fun filled geoduck planting adventures. To date over 1,400 hours of volunteer work have been logged to plant 38,000 geoducks. The successful recruitment and management of volunteers is essential to this program since installing the geoduck "condos" and planting the geoducks are but one aspect of the enhancement effort. Retrieving the condos after a year and patrolling the planted areas for human predators and polluters over the next 4 to 5 years are other important volunteer functions. Working together to support geoduck enhancement efforts has lead to increased recreational opportunity and heightened awareness of the marine environment.

RESTORING PASCAHANNA BAR—A PARTNERSHIP BETWEEN A POWER PLANT AND A STATE OYSTER HATCHERY. **T. W. Klares**, Environment Specialist, Natural Resource Management Department, Potomac Electric Power Company, 1900 Pennsylvania Ave, NW, Washington, D.C. 20068-0001, **R. E. Bohn**, Oyster Recovery Program Manager, Maryland Department of Natural Resources, Piney Point Aquaculture Center, PO Box 150, Piney Point, MD 20674.

In 1996, a group of employees from the Potomac Electric Power Company's (PEPCO) Morgantown Generating Station and the company's corporate Environment Group formed a project team with the objective of improving the productivity of a natural oyster bar. The 85-acre bar, named Pascahanna, sits just off-shore of the fossil-fueled power plant in the Potomac River. To provide oysters for the bar, company biologists—aware that state of Maryland resource managers were targeting oyster bars in lower-salinity waters such as those found in Morgantown for transplanting hatchery reared "disease-free" seed oysters—developed a partnership with one of the state's oyster hatcheries, Piney Point Aquaculture Center. Responsibilities of the partnership involved the production of seed oysters for the bar by Piney Point, and an essential adoption of the management of the 85-acre bar by the PEPCO workers. Specifically, this "adoption" included providing 1) data feedback on growth, mortality, and disease infestation; 2) manual labor at the hatchery; 3) engineering assistance; and 4) transportation of all oysters destined for Pascahanna. The first year of the partnership, 1996, saw no production of oysters at all from Piney Point due to unfavorable environmental conditions (i.e., unusually low salini-

ties). Oyster production at Piney Point boomed in 1997 with the complete renovation and upgrade of the facility. Subsequently, over 2 million oysters were placed overboard on Pascahanna bar that year. To accommodate transportation of the increased production in 1997, PEPCO also provided transportation for over 8 million oysters to other Potomac oyster bars, as well as to sites around the Chesapeake Bay for most of the 50 million oysters produced at the hatchery. The partnership continues in 1998 with an estimated 2 million oysters delivered by PEPCO for bars other than Pascahanna, and a target of planting 1 million oysters on Pascahanna later this year.

QPX A PROTISTAN PARASITE OF HARD CLAMS (*MERCENARIA MERCENARIA*) AND ITS IMPORTANCE TO REHABILITATION EFFORTS. J. N. Kraeuter and S. E. Ford Haskin Shellfish Research Laboratory, Institute for Marine and Coastal Sciences and New Jersey Agriculture Experiment Station, Rutgers University, Port Norris, NJ 08349, R. Smolowitz, Laboratory for Aquatic Animal Medicine and Pathology, University of Pennsylvania, Marine Biological Laboratory, Woods Hole, MA 02543, D. Leavitt, Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, L. M. Ragone, Calvo School of Marine Science Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

QPX, a protistan parasite has recently been associated with mortality of hard clams, *Mercenaria mercenaria*. This parasite has been found from Prince Edward Island, Canada, Cape Cod, Massachusetts, and the ocean lagoons of New Jersey and Virginia. Tissue sections of hatchery produced seed clams and hatchery clams during their first year of field growout failed to show QPX. The parasite appears in the second year of growout and can cause very high (>75%) mortalities. In 1995, clams from NJ hatcheries, being grown in Duxbury and Provincetown, MA, experienced high mortality, and heavy QPX. In late 1996, a New Jersey clam grower noted heavy mortality in stocks purchased from South Carolina in 1995. These were diagnosed to be infected with QPX. Other NJ growers have experienced mortality of SC seed purchased in 1996 and 1997. NJ seed planted in nearby plots has not experienced heavy mortality. In 1996, Virginia began a hard clam survey, and has found the parasite in a number of cultured clam plots. Virginia growers have not experienced heavy mortalities. Our current understanding of this parasite lacks many critical details, but QPX may invade clams stressed by poor growing conditions or other factors. There is an indication that genetics of particular stocks and/or latitudinal shifts of stocks may be an important risk factor. This hypothesis suggests, if rehabilitation or stock enhancement is the primary goal, that the use of seed from local stocks may be of critical long term importance, even if such seed costs more.

QUAHAUG (*MERCENARIA MERCENARIA*) STOCK RESTORATION PROJECT IN PRINCE EDWARD ISLAND, CANADA. T. Landry, M. Ouellette, and N. MacNair, Department of Fisheries and Ocean, PO Box 5030, Moncton, N.B., E1A4Y1, Canada.

Quahaug (*Mercenaria mercenaria*) production and harvesting in Prince Edward Island is continuing to be an important commercial and recreational activity. Over the past 20 years, aquaculture techniques have been evaluated in an attempt to increase the production of quahaugs, with limited success. The present project is looking at areas where small intervention in the biological cycle of this species could improve the recruitment and survival levels, similar to how desilting and shelling activities have contributed to the production of oyster. The first phase of this project started in May, 1998 and will look into the reproduction cycle of quahaug in a study bay. The natural recruitment and survival patterns in relation to habitat characteristics are also investigated and the preliminary finding will be presented.

RED AND GREEN ABALONE SEED GROWOUT FOR RESEEDING ACTIVITIES OFF POINT LOMA, CALIFORNIA. D. Lapota, G. Rosen, C. H. Liu, C. Norita, and J. Chock, Naval Space and Warfare Systems Center, Marine Environmental Quality Branch, Code D362, 53475 Strothe Road, San Diego, CA 92152-6310.

At present, there is a statewide moratorium on the harvesting of any abalone species from San Diego County north to San Francisco, CA. Harvests of the main species which comprise the fishery (the red abalone *Haliotis rufescens*, the green abalone *H. fulgens*, and the pink abalone *H. corrugata*) have plummeted to an all-time low of several hundred thousand pounds of abalone per year. Over-fishing and now several diseases have recently been identified in abalone, which are creating concern within the industry with respect to the recovery of the fishery. Presently, hatchery-bought red and green abalone seed are being cultured for eventual re-planting into the Department of the Interior Cabrillo National Monument. Abalones are being cultured in a flow-through tank system at our Biological Effects Program test facility located near the mouth of San Diego Bay and fed on a mixed diet. The seed are approximately 45 mm in size and will be transplanted when they attain the size of approximately 75-mm in size. Previous attempts at reseeded within California waters have failed for various reasons, but the present effort will use seed 3 times larger than past efforts. Reseeding success will be assessed by annual dive surveys and should help future reseeded efforts for shellfish restoration.

RESOURCE VALUATION AND BEST MONITORING STRATEGIES FOR SOFTSHELL CLAM (*MYA ARENARIA*) RESOURCES OF CHARLOTTE COUNTY, NEW BRUNSWICK, CANADA. K. L. LeBlanc, Eastern Charlotte Waterways Inc., 17 Main Street, St. George, NB, E0G 2Y0 Canada.

Softshell clams are harvested over the entire southern coast of New Brunswick. The resource is a vital part of Charlotte County's economy and the clam industry directly employs nearly 500 local diggers. Presently, many softshell clam areas in Charlotte County are closed because of poor or uncertain water and clam tissue quality. Federal agencies continue to declassify and/or conditionally close many growing areas because of decreases in monitoring effort (due to diminishing financial resources). However, harvesting in these areas might be made possible by sharing these efforts between Provincial and Federal Agencies and industry. For example, Pocologan Harbour is conditionally opened in accordance with a detailed sampling regime developed by government and industry. The harvest of clams from this traditional flat has generated more than \$1,000,000 from 1996–1998. More recently, clam digging in the conditionally opened Chamcook Harbour (through the same initiatives) generated more than \$250,000 during four weeks of harvest. More growing areas might be reclassified and reopened for digging only if such initiatives are continued. Efforts between government and industry, including community groups, will be required to guarantee the future existence of the softshell clam industry. Eastern Charlotte Waterways, Inc. (ECW) is currently developing a management plan for the monitoring of Charlotte County's coastal growing areas. In addition, future initiatives will also be taken by all significant parties to develop best management practices for the softshell clam industry as there are few presently available for the protection of this ecologically and economically important resource.

UTILIZATION OF RESTORED OYSTER REEFS BY FISHERY ORGANISMS: EXPERIMENTAL ANALYSES OF PREDATOR-PREY RELATIONSHIPS. H. S. Lenihan and G. W. Thayer, National Marine Fisheries Service, Beaufort Laboratory, Beaufort, NC 28516, J. H. Grabowski, Institute of Marine Sciences, University of North Carolina, Morehead City, NC 28557.

Oyster reef habitat is restored in Atlantic coast estuaries primarily to revive the failing oyster fishery. The goals of our project were to examine experimentally in North Carolina how species diversity and abundance of fishes and crabs, and predator-prey relationships among fishery organisms is influenced by (1) the substrate type used to restore oyster reefs, and (2) a large-scale disturbance, bottom water hypoxia. Fishes, crabs, benthic prey populations, and gut analyses were sampled on experimental reefs constructed of different materials (shell, small marl rock, large rocks) over time in Pamlico Sound. The same response variables, along with hydrographic conditions, were sampled on experimental reefs of different sizes at two water depths (3- and 6-m) in the

Neuse River before, during, and after a long-term hypoxic event. At both locations, southern flounder, pigfish, croaker, silver perch, weakfish, gag grouper, sheepshead, and blue crab were found to utilize and forage on oyster reefs. In Pamlico Sound, the most abundant fishes, pigfish, silver perch, pinfish, and flounder were found to prefer small marl reefs, where the density of benthic crustaceans (grass shrimp and amphipods) was highest. Fishes and blue crabs also appeared to control the abundance and distribution of crustaceans on reefs in the Neuse River. Bottom water hypoxia had a dramatic effect on fishery and prey organisms: hypoxia forced fishes and blue crabs onto shallow-water reefs where predation was intense and the abundance of forage species quickly declined. In general, restored oyster reefs appear to provide important habitat for a wide variety of fishery organisms and their prey. In addition, we compare the overall economic value of fishery organisms that utilize seagrass beds and natural and restored oyster reefs in Pamlico Sound and will present these findings.

USE OF WATER JETTING FOR RESTORATION OF SHELLFISH HABITAT IMPACTED BY LEAD SHOT. M. R. Liberati, E. I. duPont de Nemours and Company, Barley Mill Plaza 27, Wilmington, DE 19880, J. H. Volk, Bureau of Aquaculture and Laboratory, Connecticut Department of Agriculture, Rogers Avenue, Milford, CT 06460.

The Connecticut oyster industry has experienced tremendous growth during the past decade due in part to the implementation of culch seeding programs, and careful management of seed and harvest beds. The mouth of the Housatonic River provides a vital habitat for oyster spawning and seedling development. Very productive public and leased harvest areas are located beyond the mouth of the river in Long Island Sound. Contiguous to the oyster seed and harvest beds is a subtidal area where lead shot has accumulated from 60 years of off-shore shooting from a former gun club. A 1992 order requires the removal by mechanical dredging of an estimated 1,500 tons of lead shot contained in 400,000 cubic yards of sediment. Due to the potential negative impacts to the nearby oyster resources, alternatives to mechanical dredging were evaluated. An innovative technique to accomplish lead shot burial using water jetting was piloted to determine its effectiveness to clear the benthic zone of lead shot.

PARALLELS WITH ISSUES AND PERSPECTIVES IN ARTIFICIAL REEF RESEARCH. W. J. Lindberg, Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st St., Gainesville, FL 32653.

Oyster reef and rock reef habitats differ in many obvious respects, but as structural components of estuarine/marine ecosystems, they also offer many functional comparisons and contrasts. Practical issues pertaining to artificial reefs have afforded us opportunities to test various ecological processes, which, in turn, are

now guiding artificial reef design and placement strategies. As just one example, gag grouper (*Mycteroperca microlepis*), a top resident piscivore, is apparently capable of density-dependent habitat selection, and selects habitat patches primarily on the basis of available shelter rather than food resources. Patch reef size and spacing interact to affect gag residency times. The longest residency occurs on large, widely spaced patch reefs. And yet, growth and relative weights are greatest on smaller patch reefs, where shelter presumably limits local densities thus yielding higher per capita prey availability. Such spatial processes are important because of trophic coupling between reef and non-reef compartments of the system. An interesting comparison might be the extent to which oyster reef and rock reef habitats function as structural shelter versus sources of food for exploited species. As we construct or restore reef habitat, we ought to design the projects in accordance with the ecological processes thought to correspond to specific management objectives, and in a way that facilitates testing whether the anticipated effects are indeed manifested.

A RESOURCE-BASED METHODOLOGY TO ASSESS DOCK AND PIER IMPACTS ON PLEASANT BAY, MA.

S. L. Macfarlane, Town of Orleans Conservation Department, 19 School Road, Orleans, MA 02653. **J. Early**, Island Foundation, 589 Mill Street, Marion, MA 02738-1418. **T. Henson**, MA Coastal Zone Management, 3225 Main Street, Barnstable, MA. **T. Balog**, Town of Brewster Conservation Department, 2198 Main Street, Brewster, MA 02631, and **A. McCleennen**, Bayplan Steering Committee, 68 Evelyn's Drive, E. Harwich, MA 02633.

The preparation of a four-town resource management plan for Pleasant Bay, Cape Cod, MA required a comprehensive assessment of the present number of private piers on the bay, the probability of numerous additional piers in the future, and the potential impacts from piers on the resources of the bay. The planning group developed a methodology to account for various components relative to piers and their use. The study area was segmented into twenty six geographic subsections. Each subsection was evaluated for nine factors representing biological, physical, and human use characteristics critical to the impacts of docks and piers. The nine factors were: enclosed/open water bodies, water depth, shellfish habitat, eelgrass, fringe marsh, density of existing structures, moorings, navigational channels and recreational activity. Each of the nine factors were assigned a value of "0", ".5" or "1" where 0 indicated the least significance and 1 represented the greatest significance. Results were tabulated and mapped according to resource sensitivity. The results indicate that a significant portion of the Bay's shoreline is extremely resource sensitive. The environmental impacts from construction and use of docks and piers in these areas pose a direct threat to the extensive and fragile resources and have been deemed inappropriate for new docks and piers. Less sensitive areas may be more appropriate for construc-

tion of new docks and piers based on bay-wide criteria to be formulated once the plan is implemented.

OYSTER REEF RESTORATION IN CHESAPEAKE BAY: RECRUITMENT PATTERNS AND REEF DEVELOPMENT IN VIRGINIA. R. Mann, M. L. Luckenbach, J. Wesson, I. Bartol, J. M. Harding, M. Southworth and J. Nestlerode, Virginia Institute of Marine Science, The College of William and Mary, and Virginia Marine Resources Commission, Gloucester Point, VA 23062.

Since its programmatic inception in 1993, three dimensional reef restoration in the Virginia portion of the Chesapeake Bay has and continues to employ sites from the low salinity tributaries of the southern shore of the Potomac River (Coan River), to small "trap-type" (sensu Andrews) sub-estuaries on the western shore (Piankatank and Great Wicomico Rivers), limited sites in a large western shore sub-estuary (James River), creeks or embayments with limited watershed on the eastern shore (Pungoteague Creek) and near the mouth (Lynnhaven Bay), and a Bay mouth site at Fisherman's Island on the Eastern Shore. Structures vary from shell mounds (oyster, clams shell or a mixture) exposed at low tide both with and without supplemental broodstock additions, sediment mounds, capped with shell, and manufactured substrates (fly ash). From this cumulative data set is emerging a description of recruitment patterns at varying spatial scales, including (1) within the matrix of the surface shell layers at cm scales, (2) with depth on the intertidal - subtidal cline at cm-m scales, (3) within sub-estuaries at km scales, and (4) between sub-estuaries but within the bay at 10–100 km scales. Scales (3) and (4) also represent a salinity cline. In addition, recruitment at a limited sub set of sites can be described in temporal scales of (1) weeks within a year, and (2) up to four sequential years. An overview of these data is presented in context of longer term (decade) historical data for recruitment of oysters in these sites.

RESTORED REEFS AS AN ENHANCEMENT AND MANAGEMENT TOOL FOR SHELLFISH POPULATIONS. R. Mann, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

Odum's calculation of the value of an area of salt marsh was a significant advance in the recognition of salt marshes as valuable components of the coastal ecosystem. Data from more recent investigations of submerged aquatic vegetation (SAV) has been similarly successful in developing wide support for their restoration in coastal regions. The challenge before use is to expand the growing enthusiasm of the scientific community for oyster reef systems as an enhancement and management tool for shellfish populations to a wide acceptance of their value by the regulatory community, recreational, and commercial fishing sectors, environmental groups, and an informed general public. The contextual

problem here is daunting, in that while we as individual researchers are compiling defensible quantitative studies of individual aspects of reef ecology, we await a synthetic summary of our collective studies which has evolved sufficiently to insert reef development as a continuing discussion item in broader efforts addressing coastal and estuarine water quality, shoreline development, nutrient reduction, finfish fisheries management, and ecosystem restoration. With this goal in mind three questions will be addressed: (1) Over what spatial scale can reef enhancement effects be demonstrated (can we make a big enough impact to be taken seriously?), (2) what time frames are required to develop a sustained positive impact (can we become "players" in a reasoned time frame?), and (3) what will it cost in comparison to alternate approaches?

RECONSTRUCTION OF A NATURAL OYSTER BAR IN THE CHOPTANK RIVER USING HATCHERY PRODUCED OYSTER SEED. D. Meritt, J. Takacs, and G. Baptist, Horn Point Environmental Laboratory, University of Maryland, Cambridge, MD 21613, K. T. Paynter, Department of Zoology, University of Maryland, College Park, MD 20742, R. Pfeiffer, Oyster Recovery Partnership, Annapolis, MD 20676.

The Maryland Oyster Recovery Action Plan calls for the use of disease-free hatchery seed in the reconstruction of oyster bars in specific zones where no oyster harvesting is allowed and no Dermo or MSX-infected seed may be planted. This plan will allow experimental projects to be performed to test how quickly oyster seed will become infected in these areas and how diseases affect oysters in relatively low salinity areas over a period of years. Reconstruction of a natural oyster bar in the Choptank River was initiated in 1995 with the deposition of 100,000 bushels of dredged fossil oyster shell on a 10 acre portion of a natural bar in the Choptank River. This produced a large, hard platform on which the hatchery, held in setting tanks 4 to 10 days after settlement then moved to nursery sites in the Choptank River. After 4 to 6 weeks at the nursery sites where the spat grew to approximately 15 mm, they were planted on the prepared oyster bar. By October, 1995, the spat had grown to an average height of 28 mm. Heavy fresh-water input February–June 1996 has lowered the salinity at the site to 3 ppt which may threaten the survival of the young oysters. May sampling revealed no additional mortality due to the low salinity. Surveys will continue through 1996 to determine growth, survival and infection rates of the oysters.

THE INFLUENCE OF CREATED INTERTIDAL OYSTER REEFS ON NEKTON USE OF ADJACENT CREATED SALT MARSH HABITAT. D. L. Meyer, National Marine Fisheries Service, Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516.

Five year old created *Spartina alterniflora* marsh sites were modified in summer 1992 by oyster cultch addition to the lower intertidal fringe of randomly selected sections (cultched), while

other sections remained unaltered (non-cultched). Fauna were collected, using fyke nets, from the marsh treatments and natural (reference) marsh over two years during spring, summer and fall to examine the effect that enhancing habitat heterogeneity has on faunal marsh use. Faunal use of both cultched and non-cultched created marsh differed statistically from nearby reference marshes. After seven years, resident fish, shrimp and crab use of non-cultched marsh was frequently lower ($p < 0.05$) than reference marsh. Use of the both cultched and non-cultched created marsh by *Callinectes sapidus*, *Penaeus aztecus*, *Palaemonetes pugio*, *Lagodon rhomboides*, *Gobionellus boleosoma*, *Gobionellus boscii*, *Cyprinodon variegatus*, *Fundulus heteroclitus* and *Fundulus majalis* was significantly less than that of reference marsh. Disparities for fish and decapod abundance between created and reference treatments were more evident during summer when many species ontogenetically shift feeding patterns from plankton to epibenthic meiofauna. Prey species for marsh residents may be in lower abundance in created marsh treatments due to significantly lower particulate organic mater content compared to reference marshes. Addition of an oyster cultch fringe to the marsh was beneficial to resident marsh species use and those species with an affinity for oyster substrate, including *C. variegatus*, *F. heteroclitus*, *G. boleosoma*, *G. boscii*, *P. pugio*, and *Palaemonetes vulgaris*. The presence of the cultch may serve as a barrier to juveniles of some species, particularly *Leiostomus xanthurus*, and *C. sapidus*, which move onto the marsh early in the tidal cycle.

JAPAN'S EFFORTS TO PROMOTE NEW CONCEPTS FOR 200 MILE EXCLUSIVE ECONOMIC ZONE AND THE ROLE OF MARINO-FORUM 21. T. Morikawa, 6-4 Uchikanda 2-chome, Chiyoda-ku, Tokyo 101-004, Japan.

Until 1988, Japan had been the world's leading nation in fish production; however, the fish catch has decreased significantly. Total catch in 1996 was 7.4 million mt which was about 5 million mt less than peak of the catch in 1988 due mainly to the sharp decline in sardine landings. Fish resources in the water surrounding Japan have generally been under pressure in recent years because of the degradation of the coastal environment and the imbalance between population size and fishing effort. Nevertheless, coastal fisheries production from capture fisheries has remained at about 2 million mt over the past 30 years. This remaining at same level in coastal production was mainly a result of Japan's efforts in promoting stock enhancement and sustainable utilization of fisheries resources implemented under the "Coastal Fishing Grounds Improvement and Development" and "Sea Farming" projects. The projects, still underway, are designed to enhance the productivity of fisheries resources in the coastal waters through the creation and improvement of fishing grounds and sea farming based on the concept of restoring resources. It includes the installation of artificial fish reefs, development of suitable habitats for fishery resources enhancement, creation or restoration of seaweed beds and

tidelands, recovery or improvement of existing fishing grounds by dredging, creating water routes, and removing accumulated sludge and restoration system of non or less dissolved oxygen sea bottom layer. In recent years, new technologies have been developed and implemented by Marion-Forum 21 under the projects. These include the use of a combination of audio signal training and protective fish reefs together and feed released juvenile fish to restore and increase the population of targets species through the release of seed in suitable areas of the sea while ensuring proper conservation of existing natural stock which is called "Marine Ranching" and development of a large scale artificial upwelling-flow-generation system to increase the productivity of fishing grounds, etc. Recently, in order to improve the reliability of stable seed production, as well as to reduce labor and space requirement for mass production of marine rotifers, a continuous culture system has been developed by Marino-Forum 21. Through the "Coastal Fishing Grounds Improvement and Development" and "Sea Farming" projects taking with these new technological developments, Japan pursues responsible fisheries practices as recommended by the International Conference on Responsible Fishing.

TRENDS IN EARLY COMMUNITY DEVELOPMENT AND TROPHIC LINKS ON CONSTRUCTED OYSTER REEF.

J. A. Nestlerode, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062, **M. W. Luckenbach** and **F. X. O'Beirn**, Virginia Institute of Marine Science, College of William and Mary, Wachapreague, VA 23480.

Replicated small reef bases, constructed near the mouth of Chesapeake Bay at Fisherman's Island in the summer of 1996, are currently being monitored for the development of attached and mobile organisms on the reef surfaces and for the presence of nekton adjacent to the reefs and in nearby habitats. The reefs are composed of three different substrate materials: oyster shell, surf clam (*Spisula solidissima*) shell, and stabilized coal ash pellets and although these artificial reef communities are presumably in early stages of development, patterns in faunal abundances with respect to substrate type and tidal elevation are apparent. These findings have implications for the types of substrates and construction configurations needed to support the development of epifaunal assemblages on constructed reef habitats. The ultimate goal of this study is to use benthic and nektonic community data, along with trophic transfer data, to develop a quantitative dynamic budget model for energy flow for the reef system. This model may be used to assess ecological function of the constructed reef within the ecosystem and may lead to the development of much-needed success criteria for the evaluation of these types of constructed reef habitats in estuarine systems. Innovative sampling methods for quantifying benthic and nektonic fauna will be discussed and hypothesized trophic links between these reefs and adjacent shallow water environments will be presented.

USE OF UNDERWATER VIDEO TO MONITOR AND QUANTIFY USE OF CONSTRUCTED OYSTER REEF HABITATS BY MOBILE COMMERCIAL AND ECOLOGICALLY IMPORTANT SPECIES. **J. A. Nestlerode**, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA. 23062, **M. W. Luckenbach** and **F. X. O'Beirn**, Virginia Institute of Marine Science, College of William and Mary, Wachapreague, VA 23480.

Defining the role of oyster reefs as essential habitat for both commercially and ecologically valuable fishes and crustaceans is a crucial element in their restoration. Because of the nature of these reef systems, these habitats are difficult to sample by conventional fisheries methods. In an effort to quantify the use of reef habitats and evaluate the success of constructed reef systems, underwater video was used to compare fish use of the reef and unvegetated subtidal mudflat immediately adjacent to the reef in relation to tidal stage at Fisherman's Island, VA. Utilization of three reef area sub-habitats (reef interior, reef edge, and subtidal mudflat approximately 5 meters away from the reef edge) by nekton was monitored simultaneously at each of the three sub-habitats over an entire daylight tidal cycle. Video highlights and methods for using video in a quantitative manner will be presented.

NORTH CAROLINA'S SHELLFISH HABITAT AND ABUNDANCE MAPPING PROGRAM. **E. B. Noble** and **M. D. Marshall**, North Carolina Division of Marine Fisheries, Morehead City, NC 28557.

The North Carolina Division of Marine Fisheries shellfish mapping program maps the state's estuarine waters to locate shellfish producing areas and delineate potentially productive bottom. Oyster (*Crassostrea virginica*), clam (*Mercenaria mercenaria*), and scallop (*Argopecten irradians*) concentrations are determined by a stratified random sampling scheme. In 1987, the state's estuaries were divided into system components based on set criteria. These criteria ranked areas on shellfish habitat suitability (salinity, bottom sediments, seagrass), water quality, shellfish production, shellfish fisheries, and state management activities in the area. Twenty-four habitat types (strata) were identified to further facilitate quantitative sampling. To date, DMF has mapped over 95,000 acres of North Carolina's estuarine bottom from the Cape Fear River to the Newport River, including South River and areas in Core and Roanoke sounds. Shellfish densities are sampled in each stratum within each area and entered into a biological database. Habitat coverage is included in the state's Geographic Information System. The two databases are integrated to produce resource maps of shellfish producing areas and potentially productive bottom. Shellfish habitat and abundance maps identify and protect essential fisheries habitat, such as marsh, seagrass and oyster reefs. Information is used by coastal resource managers in the permit review process, in the designation of critical habitat, and in shell-

fish restoration and enhancement efforts. Cooperation with state and federal agencies and universities is an important program objective.

OYSTER RECRUITMENT AS A FUNCTION OF SUBSTRATE TYPE AND TIDAL ELEVATION. F. X. O'Beirn, and M. W. Luckenbach, Virginia Institute of Marine Science, College of William and Mary, Wachapreague, VA 23480. J. Nestlerode, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA.

Restoration of degraded oyster reef habitat generally begins with the addition of substrate which serves as a reef base and site for spat attachment. Remarkably, little is known about how substrate type and reef morphology affect the development of oyster populations on restored reefs. Three-dimensional, intertidal reefs were constructed near Fisherman's Island, VA: two reefs in 1995 using surf clam (*Spisula solidissima*) shell and eleven reefs in 1996 using surf clam, oyster shell and stabilized coal ash. We have monitored oyster recruitment and growth quarterly at three tidal heights (high-, mid- and low- intertidal) on each reef type since their construction. Oyster recruitment in 1995 exceeded that observed in the two subsequent years. High initial densities on the 1995 reefs decreased and stabilized at a mean of 418 oyster per m². Oyster settlement occurred on all reef types and tidal heights in 1996; however, post-settlement mortality on the surf clam and coal ash reefs exceeded that on the oyster shell reefs which remained relatively constant throughout the year (mean = 773 oysters/m²). Field observations suggest that predation accounts for most of the observed mortality and that the clam shell and coal ash reefs, which have little interstitial space, suffer greater predation. Oyster abundances were consistently greatest higher in the intertidal zone on all reefs in each year studied. The patterns observed here lead to the preliminary conclusion that the provision of spatial refugia (both intertidal and interstitial) from predation is an essential feature of successful oyster reef restoration in this region.

SHELLFISH RESOURCE DEVELOPMENT ACTIVITIES IN FLORIDA. B. D. Pierce, and J. D. Gunter, Department of Environmental Protection; Division of Marine Resources; Bureau of Marine Resource, Regulation and Development, 3900 Commonwealth Blvd, Mail Station 205, Tallahassee, FL 32399-3000.

Shellfish resource development activities are employed to increase shellfish production in 21 of the 37 shellfish harvesting areas within Florida. Restoration operations to enhance oyster production include deposition of cultch, relaying, and seed transplanting. Hard clam production has been enhanced by the establishment of high density aquaculture lease areas and other aquaculture activities. Other shellfish enhancement activities sponsored by the state include: scallop restoration activities, assisting county governments with stormwater management programs to improve water quality in shellfish harvesting areas, training county govern-

ment staff to evaluate and monitor shellfish harvesting areas, previously closed by the state due to budgetary restrictions, and cooperative programs with local, state and federal agencies that led to restoration of traditional harvest grounds previously closed due to improperly treated sewage discharged from coastal communities. Two shellfish harvesting areas (Apalachicola Bay in Franklin county and Cedar Key in Levy county) are provided as examples where resource enhancement and aquacultural activities have a profound effect with assisting fishermen displaced by the recent ban on the gill netting industry and providing shellfish harvesters with supplemental income in times of need.

REMEDIATION PROJECTS TO ADDRESS NON-POINT SOURCE POLLUTION IN THE COMOX VALLEY. O. D. Pinho, Comox Valley Project Watershed Society, #3-2401 Cliffe Ave, Box 363 Courtenay, British Columbia, V9N 2L5 Canada.

The Baynes Sound, located on the east coast of Vancouver Island in western Canada, is one of the province of British Columbia's prime shellfish culture areas (valued at \$8 million). Baynes Sound Stewardship Action Group (BSSAG) was formed in 1994 to address and remediate non-point sources of bacteriological pollution which threaten the health of the Sound. Remediation action projects for urban stormwater, agricultural runoff, and malfunctioning septic systems successfully involve dozens of funders and community volunteers. In 1996, forty community volunteers were trained to monitor bacteriological water quality at 60 storm-drains for a six month period. Sixteen of the drains were found to pose a high shellfish or human health risk (greater than 1000–5000 CFU/100 ml). The program worked with municipal governments to identify sanitary sewer cross connections. To date, over 42 sanitary sewer/storm drain cross connections have been repaired. In 1997 and 1998 an agriculture education campaign aimed at addressing pollution prevention reached 200 landowners. Hobby and commercial farmers are offered up to \$700 to assist them with remediation activities such as streamside fencing, vegetation and manure containment. Proper septic care and maintenance education workshops and pump-outs occurred in the summer of 1996 and 1997. Community volunteers distributed information to their neighbors and invited them to a "septic social / oyster barbecue" at a nearby resident's home. 2000 information packages have been distributed. Marine monitoring in the Comox Harbour (North Baynes Sound) from 1995 to 1997, indicates a slight improving trend in the frequency and magnitude of fecal coliform counts. The frequency of counts greater than 43 MPN/100 ml decreased from 1995 to 1997, however these low levels were also coincident with lower rainfall levels.

INCREASED DENSITY OF LARGE RANGIA CLAMS IN LAKE PONTCHARTRAIN AFTER BANNING SHELL DREDGING. S. W. Abadie and M. A. Poirrier, Department of Biological Sciences, University of New Orleans, Lakefront, New Orleans, LA 70148.

Rangia cuneata is a relatively large clam that is found in oligohaline areas of Atlantic and Gulf of Mexico estuaries. *Rangia* is common in Lake Pontchartrain, Louisiana, and accumulated shells supported a mining industry from 1933 to 1990. Shells were primarily used for construction of roadways, parking lots, levees and in the production of cement. Based on mean densities, large clams (> 20 mm) were abundant (95/m²) in a 1954 study, but less abundant in 1973 (44/m²), 1982 (< 1/m²) and 1984 (40/m²) studies. Because baseline and comprehensive time sequence studies were not done, it was unclear whether these differences in abundance were caused by shell dredging. Forty-five sites were sampled in 1996 and 1997 to determine whether the number of large clams had increased after shell dredging was stopped in 1990. Large clams were abundant (mean density 137/m²) at most sites, but absent in a area of about 200 km² which was affected by saltwater intrusion and hypoxic conditions from the Inner Harbor Navigation Canal (IHNC). Although large clams were absent from the eastern Lake in prior studies, the highest density (722/m²) was found in this area. Based on the current distribution and density of large clams, shell dredging had a significant impact and recovery has occurred.

OYSTER BEDS AS ESSENTIAL HABITAT FOR DECAPODS AND FISH. M. H. Posey and T. D. Alphin, Department of Biological Sciences, University of North Carolina at Wilmington, Wilmington, NC 28403, T. K. Frazer, Department of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32653, S. B. Blitch, St. Martins Marsh Aquatic Preserve Complex, Florida Department of Environmental Protection, 5990 N. Tallahassee Road, Crystal River, FL 34428.

Oysters have long been managed as a fishery, however, the habitat function of oyster beds is now receiving renewed attention. Many decapods and fish depend upon structural habitats as refuges, foraging areas, or nurseries. This function is widely recognized for seagrass beds. Yet, the absence of seagrasses throughout much of the southeastern United States and along portions of the Gulf coast, indicates the potential importance of other structural habitats in those areas. Over the past 7 years, we have examined the potential role of oyster beds as essential decapod and fish habitat, employing a combination of field observational and laboratory experimental work to determine usage patterns and mechanisms driving distributions. Sweep net and Breder trap sampling indicates that, in addition to reef residents such as mud crabs, a variety of transient species preferentially use oyster beds under at least some conditions. These include common taxa such as pinfish, grass shrimp, and juvenile blue crabs. However, oyster beds rep-

resent only one of several refuge habitats utilized by these transients and preference seems to vary spatially, temporally, and depending on landscape characteristics. We are currently studying regional patterns of juvenile blue crab use of oyster beds in the presence and absence of other structural habitats to better understand some of these modifying parameters for an important commercial species. If oyster beds represent an essential, or even alternative habitat, this emphasizes the need for management of these systems even in locations where shell fishing is not feasible.

MODELING THE MSX PARASITE IN EASTERN OYSTER (*Crassostrea virginica*) POPULATIONS: CHESAPEAKE BAY AND DELAWARE BAY COMPARISONS. E. N. Powell and S. Ford, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ, 08349, J. M. Klinck and E. E. Hofmann, CCPO, Crittenton Hall, Old Dominion University, Norfolk, VA 23529, S. Jordan, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654.

A series of simulations was done with a coupled oyster-*Haplosporidium nelsoni* model to investigate the processes controlling the prevalence and intensity of the MSX disease in oyster populations in Chesapeake and Delaware Bays. The host-parasite model was initially applied to Delaware Bay where it accurately simulated the observed seasonal *H. nelsoni* cycles and consequent oyster mortality. Migration of the host-parasite model to Chesapeake Bay required only two modifications, which indicates the robustness of the model. The first was a change in the background concentration of *H. nelsoni* spores in the spore sub-model, with a lower value needed for the Chesapeake Bay to match observations. It is believed that this modification reflects different water residence times in the two Bays. The second modification consisted of incorporating salinity-dependence for *H. nelsoni* sporulation that is specific to each Bay. This is thought to account for a possible secondary host for *H. nelsoni* that is responding to ambient conditions in each Bay. Using environmental conditions characteristic of Delaware and Chesapeake Bay, the model was able to accurately simulate the timing of *H. nelsoni* infections in oysters and along-Bay gradient in MSX infection intensity and prevalence. These results show clearly the need for environmentally-based management of diseased oyster populations.

ANALYSIS OF GENETIC VARIATION IN THE OYSTER PATHOGEN *PERKINSUS MARINUS*. K. S. Reece and J. E. Graves, Virginia Institute of Marine Science, College of William and Mary, PO Box 1346, Gloucester Point, VA 23062, D. Bushek and K. L. Hudson, Baruch Marine Field Laboratory, Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, PO Box 1630, Georgetown, SC 29442.

Perkinsus marinus is a major pathogen of the eastern oyster *Crassostrea virginica*. Recently developed in vitro culture and cloning methods have facilitated molecular genetic analyses of *P.*

marinus. We examined the genetic relatedness of *P. marinus* in vitro cultures, and the clonal composition of cultures established from infected oysters collected throughout the US range of the parasite. Genetic relatedness of 59 geographic isolates and 34 monoclonal cultures derived from these isolates was examined at six polymorphic nuclear loci by restriction fragment length polymorphism analysis. Distance analyses indicated two major groups. One cluster was composed predominately of isolates from the mid-Atlantic and northeastern coastal areas of the United States. The other group was dominated by isolates from southeastern Atlantic and Gulf coast regions. Polymorphic simple sequence repeat regions (microsatellites) of the *P. marinus* genome are being identified and will be utilized along with polymorphic single-copy nuclear loci to examine the population genetic structure of *P. marinus*. DNA sequence analysis of alleles at an anonymous nuclear locus in heterozygous monoclonal cultures suggested that DNA recombination may be occurring in cultured *P. marinus* cells.

SO HOW DO YOU EVALUATE A SHELLFISH RESTORATION PROGRAM? **R. J. Rhodes**, South Carolina Marine Resources Center, PO Box 12559, Charleston, SC 29422-2559.

Shellfish stocking and other shellfish enhancement programs are often predicated on the notion that the wise application of research and technology can improve or restore shellfish stocks. Although seeking remedies for restoring shellfish resources is critical, it is also important to estimate monetized costs and benefits of proposed and/or existing restoration programs. Explicit costs for shellfish restoration programs may include operating expenses and capital for specialized equipment and facilities. A shellfish restoration program may also have significant implicit or opportunity costs because the program has or will use resources (e.g., capital, skills, etc.) that could be employed in other activities. Measuring economic benefits generated from shellfish restoration programs may include the use of methods to estimate the "non-market" value that recreational harvesters place on environmental goods. These methods are usually oriented toward estimating demand for particular species and/or sites. These demand estimates often address "net willingness to pay" by recreational harvesters and others that are attributable to improved or restored shellfish stocks. Usually a cost and benefit analysis (CBA) of a restoration program needs to be evaluated over a multi-year period. Consequently, discounting is used to translate future dollars into equivalent present value (PV) dollars, which allows a comparison of all costs and benefits over a given time period. The resulting information (e.g., PV of benefits minus PV of costs) can be used to compare various restoration alternatives or scenarios. If it is judged that the overall benefits derived from a feasible range of restoration alternatives are about the same, then a CBA really becomes a cost-effectiveness analysis to estimate the least-cost restoration program. An example of a shellfish restoration CBA will be discussed along with some common misconceptions regarding CBA.

AN UPDATE ON SHELLFISH RESTORATION PROJECTS IN RHODE ISLAND. **M. A. Rice**, Dept of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, R.I. 02881. **A. R. Ganz**, Rhode Island DEM, Div. of Fish and Wildlife, Coastal Fisheries Laboratory, Succotash Road, Wakefield, RI 02879.

Preception of declining shellfisheries in Rhode Island has sparked heightened interest in restoration of shellfisheries and shellfish habitat. Past efforts in shellfishery enhancement has been through the establishment of "spawner sanctuaries" and a state shellfish relay program. Notable programs include scallop restoration in the coastal ponds in the 1970s and a relay program in Greenwich Bay since 1981. Although relays continue due to their popularity among shellfishermen, a number of aquaculture-based restoration initiatives have recently emerged and the notion that successful shellfish restoration requires a steady supply of larger, predator-resistant seed is understood by most. Two projects are underway using floating upwellers to produce seed for shellfish restoration work including one at URI. Shellfish restoration has been identified as an instructional tool at the secondary level. The *Rhode Island Public Benefit Aquaculture Project* is a collaborative project coordinated by the RI Seafood Council and the Greater RI Regional Employment and Training Board with partners from the shellfishing and marine trade industries, universities, and secondary and technical schools. Students are trained in the building and operation of floating upwellers for rearing *Mercenaria mercenaria* seed destined for planting in public shellfishing grounds. Efforts are underway at URI to assess the economic viability of shellfish restoration projects in Rhode Island based upon our local growth and natural mortality rates. Current related proposals include state legislation (H-8816) introducing a framework for private entrepreneurial efforts in aquatic habitat restoration, and the establishment of a shellfish restoration hatchery in Portsmouth, RI by the *Waterworks Group*. This is publication 3650 of CELS, University of Rhode Island.

THE IMPACTS OF URBANIZATION ON SHELLFISH HARVESTING WATERS: DEVELOPMENT OF TECHNIQUES TO IDENTIFY COLIFORM POLLUTION SOURCES. **G. I. Scott, L. Webster, B. Thompson, W. Ellenberg** and **P. Comar**, NOAA, NOS, Charleston Center for Environmental Health and Biomolecular Research, Charleston, SC 29412. **G. P. Richards**, US Department of Agriculture, Microbial Food Safety Research Unit at Delaware State University, Dover, DE.

Urbanization of upland areas adjacent to estuarine ecosystems has resulted in significant inputs of bacterial and chemical contaminants in salt marsh ecosystems of the southeastern U.S. During the pioneering states of urban development, human waste disposal needs are met by use of septic tank based technology. As urban development proceeds and critical carrying capacity for human population density is reached, significant inputs of bacterial pollution from septic tank discharges into estuarine ecosystems

may result), often causing closure of shellfish harvesting waters due to the presence of pathogenic bacterial /viral pollution. To address this problem of bacterial contamination from human waste associated with coastal urbanization, the Urbanization in Southeast Estuarine Systems (USES) Study has evaluated the effects of human encroachment on estuarine surface water quality and oyster health. Two estuarine ecosystems were chosen for study: North Inlet (NI), a pristine estuary which is a National Estuarine Research Reserve site, and Murrells Inlet (MI), one of the most urbanized coastal areas in the state of South Carolina (based upon population densities > 625/sq. mile). Bacterial indicators were measured in surface waters and oysters. Results indicated that a total of 67% of the surface water monitoring stations in MI exceeded the SA water quality criteria for fecal coliform bacteria (13/100ml) compared to only 33% of the stations in NI. Poor water quality stations in MI were associated with high densities of septic tanks in close proximity to the estuary and other urban activities (marinas, boat landings, and roadways). GIS overlays and statistical analysis indicated that regions in MI with high levels of PAHs, near roadways and marinas, also had concomitant high fecal coliform bacteria densities. Fecal coliform bacterial biotyping of surface waters indicated there were significant differences in the speciation of coliform positive species in surface waters of MI and NI. In urbanized MI, there was a greater occurrence of *E. coli* bacteria, fewer stations which were coliform negative and a reduced number of bacterial species comprising the coliform group, particularly soil sorbed microbes of the Pseudomonid family. Fecal coliform bacterial biotyping of oysters indicated that unlike results of surface waters, there were no significant differences in the speciation of coliform positive species in oysters from MI and NI. Study of oyster disease and *Vibrio* bacteria interactions indicated that oysters parasitized by *Perkinsus marinus* as a result of the higher salinity waters in MI estuary, had a greater portion of *Vibrio vulnificus* (MI = 77.3% versus NI = 34.1%) and *Vibrio parahaemolyticus* (MI = 77.6% versus 53.2%) bacteria distributed within internal tissues (gonad, digestive diverticulum, and adductor muscle) than in more external tissues (gills, mantle, and labial palps). There were no significant differences between fecal and total coliform distributions in NI and MI oysters, as the majority of the bacterial burden was found in internal tissues. These findings clearly indicate that fecal coliform bacteria pollution is associated with urbanization and that closure of shellfish harvesting waters may be perhaps the most significant, quantifiable impact from urbanization.

OYSTER REEF BROODSTOCK ENHANCEMENT AS A MECHANISM FOR RAPID OYSTER REEF REPLENISHMENT. M. Southworth and R. Mann, College of William and Mary, Virginia Institute of Marine Science, PO Box 1346, Gloucester Point, VA 23062.

Natural oyster populations in the Chesapeake Bay have become severely depleted due to a combination of overfishing and disease. Replenishment programs in the form of artificial reefs are cur-

rently in effect throughout most of the Virginia portion of the Chesapeake Bay. Shell Bar reef, built in the Great Wicomico River, Virginia in 1996 was supplemented with reproductively active broodstock oysters from Tangier and Pocomoke Sounds. The Great Wicomico River was historically a high seed producing river, but production has decreased in recent years. Oyster larval concentrations (plankton tows), gonad development, and circulation data were collected in the river throughout the 1997 reproductive season. The broodstock oysters spawned from mid-June through mid-August, with a peak occurring from mid-June through mid-July. Larval concentrations were several orders of magnitude higher than the highest reported in the literature over the past 25 years. Larvae were significantly more abundant on the flood tidal stage, suggesting some vertical migration with the changing tide, thus aiding in their retention in the system. Settlement of larvae on shellstrings and on bottom substrate, was higher than in recent years. The most abundant settlement occurred near the reef and upriver of the reef. Circulation patterns observed are favorable for local retention of larvae in the system. Reef building, and subsequent transplants of broodstock onto these reefs, can be an effective management option provided the circulation patterns of the system are similar to the Great Wicomico.

RESTORATION OF THE NEW ZEALAND LITTLE NECKED CLAM, *AUSTROVENUS STUTCHBURYI*. M. J. Stewart and R. G. Creese, Leigh Marine Laboratory, University of Auckland, PO Box 349, Warkworth, New Zealand.

Austrovenus stutchburyi, is a shallow-burrowing, filter-feeding clam, found in sheltered soft-shore intertidal habitats around New Zealand. Adult clams have an average shell length of 30–40 mm. Population of *A. stutchburyi* are vulnerable to pollution, increased sedimentation and overharvesting. Consequently, this popular resource has declined at many locations throughout New Zealand. The decline of soft shore bivalves has only recently been recognized in New Zealand. Therefore, restoration is a novel technique for New Zealand, despite being increasingly used overseas. This research investigates the potential for restoration of *A. stutchburyi*, through studies of the ecology of *A. stutchburyi*, including manipulative field experiments to assess movement patterns, predation rates and responses to translocation. Due to the inherent difficulties in tagging and relocating infaunal bivalves, a relatively new method has been trialed. Clams are tagged with small aluminum discs, enabling relation using a metal detector. Tag loss varies between treatment types, being highest for small densely packed clams. Over three sites, mean tag loss across all treatments was $8.8\% \pm 2.1\%$, after four months. This is likely to be an overestimate, as only a subsample of individuals has been recaptured. Laboratory studies show no significant difference in survival or growth between tagged and untagged clams. Ability to rebury was not affected by tags. All tagged and untagged individuals burrowed

within 24 hours of being placed in tanks. Studies previously thought logistically difficult are now feasible with this technique. Results from these studies and results evaluating this technique for monitoring enhancement will be presented.

THE GULF COAST OYSTER INDUSTRY PROGRAM: AN INITIATIVE TO ADDRESS INDUSTRY'S RESEARCH NEEDS. J. Supan, Louisiana Sea Grant College Program, 107 Sea Grant Shop, Baton Rouge, LA 70803.

The Gulf Oyster Industry Program (GOIP) evolved as a regional research program in response to petitions from the Louisiana Oyster Task Force and Gulf Oyster Industry Council. These organizations sought long-term research-based assistance from Sea Grant to help the Gulf oyster industry reach its full economic potential. Oyster producers in the Gulf states face myriad problems associated with (a) the occurrence of opportunistic bacteria, especially *Vibrio vulnificus*, in oyster growing waters; (b) multiple use conflicts in traditional oyster grounds, especially those associated with coastal restoration projects; (c) pollution impacts from upstream urban and industry development, nearby recreational camps, and oil production facilities; (d) depletion of harvestable stocks by oyster predators, competitors and diseases; (e) oyster ground closures occasioned by outbreaks of toxic marine algae; (f) uncertainty about long-term stability of oyster leasing policies; and (g) declining profitability that stems from a litany of conditions: declines in seasonal meat yields, a changing work force, additional product harvesting and handling regulations, limited technological options, and the like. The GOIP proposal solicitation was conducted as a nationwide competition for research funds in accordance with National Sea Grant policy and legislative mandate. An Industry Advisory Panel (IAP) of Gulf oyster harvesters and processors spanning the five-state Gulf region identified specific needs to provide a framework for setting research priorities. The IAP, with two members from a Scientific Review Panel (SRP), also determined which preliminary proposals would be selected for development as full proposals. Eighteen full proposals totaling \$1.1 million were selected from thirty-five preliminary proposals totaling \$3.9 million in requested funds during the program's inaugural year. These were circulated to peer scientists for detailed reviews; at least three peer reviews for each proposal. Finally, the SRP, comprised of six scientists who are generally familiar with contemporary oyster research, and two members from the IAP, selected eleven proposals based on scientific merit and written peer reviews. Proposals dealing with human pathogens, oyster disease, labor and mechanization, point-source pollution, hatcheries and genetics, harmful algal blooms, and coastal restoration were selected to receive first year funds. Projects will be funded in Virginia, North Carolina, Florida, Alabama, Mississippi, Louisiana, Texas, and California. The GOIP Steering Committee, consisting of Gulf region Sea Grant Directors, and representatives of the National Sea Grant Office, the National Fisheries Institute, the

Louisiana Oyster Task Force and the Gulf Oyster Industry Council, provided guidance on program implementation and gave final approval of project awards.

MIND YOUR E'S AND C'S! AN EFFECTIVE APPROACH TO SHELLFISH RESTORATION. K. A. Tammi and W. H. Turner, The Water Works Group, Inc. PO Box 197, Westport Point, MA 02791.

The Water Works Group, Inc. is a non-profit organization dedicated to shellfish restoration, particularly of bay scallops, *Argopecten irradians* in Massachusetts waters. In 1993, we initiated the Bay Scallop Restoration Project (BSRP) in the Westport River as a means to not only restore this prized shellfish, but also to evoke public interest in water quality woes plaguing the river. Presently, the BSRP is about to begin its sixth year of educational programs and shellfish research. During this time, our research has added to the scientific knowledge on scallop biology and ecology. The progress of the BSRP research has been significant, involving over 4,000 students, 600,000 volunteer hours, 24 full-time interns, and 3 successful graduate student research projects; these efforts culminated in a substantial bay scallop harvest for the Town of Westport in 1996. This restoration endeavor experienced its share of difficulties. Having touted the four E's: Education, Economics, Enthusiasm, and Environment to muster community support, we thoroughly understood that grassroots shellfish restoration was also about the four C's: Community, Cooperation, Coordination, and Commitment. This discussion will focus on the political lessons learned while embarking on the BSRP. The importance of shellfish restoration is a topic that cannot be addressed by one group or governmental agency alone. A well coordinated, cooperative endeavor formulated at the community level is needed to be effective. Without these components the ultimate goal can become buried in politics. However, if executed properly, grassroots shellfish restoration can produce real economic benefits with scientific merit.

SHELLFISH STOCK ENHANCEMENT AND TRAINING EFFORTS AT THE CORNELL COOPERATIVE EXTENSION (SUFFOLK COUNTY) MARINE CENTER. K. W. Tetrault, G. J. Rivara, and R. M. Patricio, Cornell Cooperative Extension-Suffolk County Marine Program, 3690 Cedar Beach Road, Southold, NY 11971.

The Marine Center at Cornell Cooperative Extension of Suffolk County, New York has maintained a shellfish hatchery for the past eight years. The principal goal of the facility has been to provide seed clams, oysters and bay scallops to local townships for the enhancement of diminishing supplies on once prolific shellfish

beds. In recent years, the Cornell hatchery has been involved in numerous training programs which have supplied local baymen with the gear, seed and information necessary for culturing shellfish to market size. In addition, training in shellfish aquaculture has been offered to college and high school interns. Tours of the hatchery educate members of the local community in the principles of aquaculture and techniques in shellfish restoration. Staff on the aquaculture team are available to any individuals interested in culturing shellfish and provide a clearing house for information on hatchery, nursery and growout techniques. The facility affords itself the luxury of experimentation often not available to commercial ventures and can pass the results, both negative and positive, to the industry. Primary research efforts include the following: Use of high lipid strains of algae and their effects on larval and post-set growth and survival; Design and use of axial flow upwellers; Plating techniques and stocking density on the survival of the northern quahog; Gear and maintenance protocol necessary for the winter survival of bay scallops. Bay scallops are of particular concern due to the near total collapse of the stocks following "brown tide" events.

UTILIZATION OF RUTGERS RESISTANT OYSTER STOCKS TO ESTABLISH SUCCESSFUL NEW JERSEY OYSTER FARM. **S. M. Tweed**, Rutgers Cooperative Extension of Cape May County, 4 Moore Road, Cape May Court House, NJ 08210.

New Jersey oyster production has severely declined as a result of MSX and Dermo infections in natural populations. Researchers at the Rutgers' Haskin Shellfish Research Laboratory, with support from the Sea Grant Program and the State of New Jersey, have developed and bred disease resistant American oyster stocks (*Crassostrea virginica*) for more than 30 years. In 1996, a shellfish company, Atlantic Capes Fisheries, became the first company to attempt to commercial production of these resistant oysters in New Jersey. Development of the oyster farm, coordinated by the Sea Grant Marine Advisory Service Agent, included adapting rack and bag and Taylor float techniques for conditions in Cape May County and training personnel in field grow out and data collection. Oyster stocks consisted of highly selected resistant lines from New England stocks and old Delaware Bay stocks. Growth and survival of these stocks were compared at three grow out areas: Great Sound, Cape May Harbor and Delaware Bay. Growth and survival rates from these areas were used to develop a successful farm production strategy and enterprise for a New Jersey oyster farm. With successful training and production strategy, the farm has grown from 65,000 oysters in 1996 to over one million oysters in 1998. Additional support for the oyster farm was provided by a Technology Transfer Grant from the New Jersey Commission on Science and Technology.

ANTIOXIDANT ENZYMES, POTENTIAL VIRULENT FACTORS, IN DIFFERENT STRAINS OF THE OYSTER PROTOZOAN PARASITE, *PERKINSUS MARINUS*. **F.-L. E. Chu**, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062, **A. K. Volety**, NRC, EPA, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561, **S. Armknecht**, Orbital Image Corp, 21700 Atlantic Blvd, Dulles, VA 20166.

The oyster protozoan parasite, *Perkinsus marinus*, is one of the two important parasites causing severe mortality in the eastern oysters (*Crassostrea virginica*) on the US east coast. Our recent study suggests that *P. marinus* cells and its extracellular products (ECP) could scavenge the reactive oxygen intermediates produced by oyster hemocytes or inhibit their production. The parasites' acid phosphatase (AP), superoxide dismutase (SOD), and other antioxidant enzymes are believed to play a role in scavenging or inhibiting hosts' respiratory burst. Recent studies also suggest that the virulence of *P. marinus* vary with strains. The extra- and intracellular activities of AP, SOD, catalase, and glutathione peroxidase (GP) were examined in six different *P. marinus* strains/isolates, i.e., Delaware Bay, New Jersey (DB-NJ), Mohjack Bay, Virginia (MB-VA), Barataria Bay, Louisiana (BB-LA), Laguna Madre, Texas (LM-TX), Oxford, Maryland (OX-MD), and York River, Virginia (YR-VA). It was found that no catalase or GP was detected in *P. marinus* and its ECP. The YR-VA strain has significantly higher extracellular AP activities (units/mg cell protein) than all other strains. Intracellular AP activity was low (1.0 unit/mg total cell protein) in all strains. LM-TX strain had the greatest intracellular AP activity. The mean SOD activity (ng SOD/mg total cell protein) was higher in the YR-VA strain, but statistically insignificant from the other strains. SOD activity was detected only in the culture media of 97, and 114 days old *P. marinus* culture. Results will be reported and discussed in relation to the virulence of this parasite.

PROGRESSION OF DISEASES CAUSED BY THE OYSTER PARASITES, *PERKINSUS MARINUS* AND *HAPLOSPORIDIUM NELSONI*, IN *CRASSOSTREA VIRGINICA* ON CONSTRUCTED INTERTIDAL REEFS. **A. K. Volety**, National Research Council, U.S. Environmental Protection Agency, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561, **F. O. Perkins**, University of Hawaii at Manoa, 105 Bachmann Hall, 2444 Dole Street, Honolulu, HI 96822, **A. R. Mann**, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, PO Box 1346, Gloucester Point, VA 23062, **P. R. Hershberg**, Meteorology Department, Florida State University, Tallahassee, FL 32306.

The progression of diseases caused by the oyster parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*, were evaluated by periodic sampling (May 1994–Dec 1995) of oysters, *Crassostrea*

virginica, that set on an artificial reef located in the Piankatank River, Virginia, in August 1993. The infections observed were recorded as a function of 1) prevalence and intensity, 2) oyster size and age, and 3) depth below mean low water at which the host oyster was found on the reef. Only a very small (insignificant) number of oysters were infected with the two species of pathogens on the oyster reef during the first eleven months of life. In the second year of oyster life, epizootiological patterns of disease development followed temperature and salinity trends. Oysters at residence depths = 45 cm below mean low water exhibited significantly ($P < 0.0001$) higher prevalence and intensity of infections than oysters at depths = 90 cm. In contrast, oysters at residence depths = 90 cm had significantly higher growth rates ($P < 0.05$) than those at = 45 cm. However, size differences were not significant ($P > 0.05$) at the end of the study. Results from the current study may be used in managing oyster fisheries on natural or artificial reefs.

GEORGIA BASIN INITIATIVE - CLEAN WATER ACTION PLAN. D. B. Walker, Environment Canada, 224 West Esplanade, North Vancouver, British Columbia, V7M 3H7, Canada.

The Georgia Basin is a large body of water located on the Pacific coast of Canada in the southwestern region of the Province of British Columbia. The basin is bounded on the west by Vancouver Island and on the south by State of Washington and Puget Sound. On the eastern shores of the basin are the City of Vancouver and to the north the waters of Desolation Sound. Rapid population growth is placing stresses on existing source of water and degrading aquatic ecosystems through pollution from non-point, urban and agricultural runoff, sewage, combined sewer/storm overflows and industrial discharges. The Georgia Basin Initiative is a five year action plan that will focus efforts to reduce/eliminate significant sources of pollution both point and non-point to the basin. Efforts will also concentrate on mitigating impacts of increased growth and urbanization on aquatic ecosystems; reduce water consumption through conservation and reuse; and take preventative and protective measures to ensure health and sustainability of the basins ecosystems are not affected by harmful substances. One of the goals of the action plan will address water quality in shellfish growing/harvesting areas with the result of re-opening 25% of areas currently closed to harvesting. To achieve this result will require partnering with all levels of government, the industry and most importantly the community. Environment Canada has undertaken this initiative as part of a long term strategy to make sustainable development a reality in Canada in order to help present and future generations of Canadians.

RESTORING OYSTER REEFS IN ALABAMA: A PROGRAM TO ENHANCE SPORT FISHING AND INCREASE OYSTER PRODUCTION. R. K. Wallace, Auburn University Marine Extension and Research Center, 4170 Commanders Drive, Mobile, AL 36615, D. B. Rouse, Auburn University, Department of Fisheries and Allied Aquaculture, Auburn, AL, M. Van Hoose, Alabama Department of Conservation and Natural Resources, Marine Resources Division, Dauphin Island, AL.

Portions of three unproductive oyster reefs totaling 30 ha in Mobile Bay are being enhanced to provide both angling opportunities and to re-establish oyster production. Reef areas were marked with pilings and encircled by a low (1 m), irregular line of concrete rubble to protect the remaining cultch material and to create structure for fishing with Wallop-Breaux funding. The three reefs and a productive reef were surveyed and assessed for standing crops of live oysters and available cultch to establish baseline data prior to additional restoration efforts. One of the unproductive reef areas is the subject of experiments to test hypotheses relative to restoration. Hatchery produced oysters (spat and seed) were placed on the existing bottom and on oyster shell pads constructed 20 and 40 cm above bottom to determine relative growth and survival. Continuous recording instruments are monitoring oxygen at two levels (bottom and 40 cm) and spat set is being checked at all three sites. The largest reef, which was productive as recently as 10 years ago, is scheduled to be planted half in oyster shell and half in limestone while the remaining two reefs will be planted based on findings from the experiments. These two reefs, which were not well known to anglers prior to marking and addition of structure, are already being heavily utilized and anecdotal reports on fishing success have been good.

OYSTER REEF RESTORATION AND THE MANAGEMENT OF OYSTER BROODSTOCK SANCTUARIES IN VIRGINIA. J. A. Wesson, Virginia Marine Resources Commission, Conservation and Replenishment Division, PO Box 756, Newport News, VA 23607.

Fifteen three-dimensional oyster reefs have been reconstructed since 1993, primarily with natural shell material. Initial intentions were to allow natural settlement to populate the reefs with the assumption that the improved habitat would impart some disease resistance. Higher settlement and faster growth rates have been observed on most reefs. In disease endemic areas, populations of small oysters increased to levels comparable to the best bars in the James River, but disease mortality has slowed the accumulation of large oysters. In 1996, large, presumably disease tolerant oysters were harvested from an open, low density area in Tangier Sound, and relocated at higher densities onto a constructed reef in the Great Wicomico River which is a "trap-type" estuary. The following summer a 10 to 200 fold increase in spatset was observed throughout an area 6 miles surrounding this reef and later 50 thousand bushels of seed oysters were moved from this river to

repopulate other areas. Similar increases in spatset have occurred in several other small rivers where large oysters have slowly accumulated following several years of harvest closure. The creation of optimal, three-dimensional reef habitat with or without the addition of broodstock and the protection of oyster populations until enough large, highly fecund oysters have accumulated to facilitate dependable spatsets appears to be the quickest way to sustainable oyster populations in Virginia. Thereafter, strategically placed "sink" or harvest areas can be intermixed with the broodstock areas to allow sustainable use of oysters by industry.

JUMP RUN CREEK SHELLFISH RESTORATION PROJECT. **N. M. White**, Landscape Architecture, Box 7701, NCSU, Raleigh, NC 27695. **D. E. Line**, NCSU Water Quality Group, Box 7637, Raleigh, NC 27695. **J. D. Potts**, NC-DENR - Shellfish Sanitation Section, PO Box 769, Morehead City, NC 28557. **W. Kirby-Smith**, Duke Marine Lab, 111 Pivers Island Road, Beaufort, NC 28516. **B. Doll**, NC Sea Grant, Box 8605, NCSU, Raleigh, NC 27695. **W. F. Hunt**, Biological and Agricultural Engineering, Box 7625, NCSU, Raleigh, NC 27695.

The objective of this multi-agency project is to 1) quantify the effects of land use change on shellfish closures and 2) assess techniques that can be used mitigate those impacts. This report is based on preliminary analyses conducted over the last 18 months. The project focuses on the 800-acre watershed in Carteret County, NC that is the drainage for Jump Run Creek. Bacterial data from 1970 through 1998 indicate little change in overall loading since 1974, which is when closure management began. Recent grab sample data from the tributaries indicate high levels of bacteria during storm events and moderate levels during dry weather. The majority of the loading is coming from the portion of the watershed draining an older, medium density neighborhood (single family houses) and a trailer park. A door-to-door survey found two malfunctioning septic systems, more than 100 pets, and the presence of wildlife. Change analysis of land use/land cover shows hydrologic modifications were instituted in the 1970's. Dye studies confirm that water moves through the watershed in hours indicating that bacterial mortality is insignificant. Future analyses include ground water sampling, automated storm water monitoring, and DNA tracking of fecal sources. Planned mitigation practices will include riparian buffer restoration, stormwater wetland, bio-retention, peat filters, and education. Locations and sizing of practices will be determined through GIS-based hydrologic analysis of the watershed in conjunction with a community-design/educational approach involving neighborhood citizens.

THE GENETIC EVALUATION OF BAY SCALLOP ENHANCEMENT SUCCESS ON THE GULF COAST OF FLORIDA. **A. E. Wilbur** and **T. M. Bert**, Florida Marine Research Institute, 100 Eighth Ave S.E., St. Petersburg, FL 33701.

In recent years, the bay scallop, *Argopecten irradians*, has been severely depleted over a substantial portion of its range in Florida. Overfishing, habitat degradation, and toxic dinoflagellate blooms have been cited as contributing factors in the collapse of many populations in west-central Florida, but the precise cause is poorly understood. The persistence of low scallop abundance and recruitment in these areas suggests that local populations may depend upon larval retention and self-seeding to maintain densities over the short term, and that reduced larval supply in these areas (because of low abundances) may be slowing recovery. To address this, Florida's Department of Environmental Protection initiated an enhancement project on the west-central coast of Florida in 1997. Hatchery-produced scallops, derived from broodstock collected from the enhancement site, were planted in bottom cages to create spawning aggregates in an effort to enhance local larval supply and recruitment. Evaluating the success of these spawning aggregates in increasing local larval supply and subsequent recruitment, requires a mechanism for quantifying the proportion of new recruits that are the offspring of the hatchery-produced scallops. We are developing genetic markers (mtDNA, microsatellites and introns) to distinguish between offspring resulting from the hatchery scallops and those produced by wild scallops. This paper will evaluate the efficacy of these genetic tags in monitoring the success of scallop enhancement.

CAN GOVERNMENT - PRIVATE SECTOR PARTNERSHIPS WORK FOR RESOURCE RESTORATION? IF NOT, WHY NOT? **M. D. Willinsky**, Coastal Engineering, Inc., 1290 Bay Dale Drive, Arnold, MD 21012. **D. L. Leonard**, Director of Fisheries, DNR-MD., 580 Taylor Ave, Annapolis, MD 21401. **M. Gluis**, Southern Shellfish Ltd., Adelaide, S. Australia.

The oysters dramatic decline in Chesapeake Bay was caused by multiple factors: severe under reporting of oyster landings, paternalistic relationships between watermen and government, where watermen hold the political power base, incidence of MSX and Dermo, use of disease as an excuse for inaction, failure to protect broodstock, use of dredged fossil shell as cultch, payment of watermen to move diseased spat and general mistrust between watermen, non profits, university, government and private sector. A Round Table Action Plan to recover the oyster population was tabled in 1992. Some recommendations were adopted, but no noticeable improvement in the oyster population occurred. In 1996, a new Director of Fisheries provided needed leadership. Team building between government, outside experts, university, non profits and watermen led to a plan to convert an abandoned State property

into a cost effective, large scale oyster settling facility. Coastal Engineering, a company that designs, builds and operates aquaculture production facilities built and managed the project. The facility was phenomenally successful achieving settlement rates 10 to 35 times greater than previously while producing 15 times the predicted number of oysters. The team scrambled with its success developing novel nursery techniques that resulted in rapid growth, high survival and dramatically lower disease incidence. Despite

these results, once private sector project leadership ended, the partnership foundered and government moved to dump the oysters. Watermen, business and government barely recovered the project. Was this a case of obvious success, engendering distrust and jealousy? Don't we all share a common goal or do different agendas, infighting and struggling over dollars destroy successful programs? Technical details, teamwork building and government-private sector interactions will be addressed.

Erratum

Rugolo, L. J., Knotts, K. S., Lange, A. M., Crecco, V. A. (1998) Stock assessment of Chesapeake Bay Blue Crab (*Callinectes sapidus* Rathbun). *J. Shellfish Res.* 17(2):493–517.

The above mentioned article was originally published in a previous issue of the journal. Because of a large number of printer errors, the corrected version of the article is being printed again in this issue. The printer regrets the errors.

STOCK ASSESSMENT OF CHESAPEAKE BAY BLUE CRAB (*CALLINECTES SAPIDUS* RATHBUN)

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ABSTRACT This stock assessment stemmed from concerns about declines in Chesapeake Bay blue crab commercial yield, survey abundance indices, and economic performance in the 1990s. Blue crab vital rates and life-history characteristics were described. A risk-averse approach was adopted for parameter estimation. Extant (50 y) fisheries-independent and dependent data were compiled. Current spawning stock biomass was at moderate levels, as compared to historical maxima; total stock biomass was at long-term average levels since 1956. Juvenile recruitment has been variable since the 1950s. The stock is in long-term dynamic equilibrium, demonstrating variable abundance as expected in a strongly r-selected species. No evidence of a persistent stock decline was found. The decade of the 1980s was a period of above average abundance; the population modulated to average levels thereafter. Maryland commercial catch per unit effort (CPUE) has remained fairly stable from 1982 to 1995. Baywide yield varied without trend since 1945, cycling around the long-term mean with peaks in 1950, 1966, 1981, and 1993 and troughs in the mid-1950s and late 1970s to early 1980s. Baywide data since 1945 demonstrated a fivefold increase in directed effort (f) and an exponential decline in CPUE: rapidly from 1947 to 1967, and without trend since 1970. The stock has supported maximum sustainable yield (MSY) = $37\text{--}38.5 \times 10^6$ kg since 1945 with no observed change in the ability of the stock to replace itself or to provide historical average yield. We judged the stock to be moderately to fully exploited at average levels of abundance. Stock-recruitment (S-R) analysis revealed weak relationships. Density-independent effects accounted for little of the variability in the S-R model. Exploitation rates for 1956 to 1996 ranged from 30–45% for peeler/soft crabs and 40–55% for hard crabs. Fishing mortality (F) ranged largely between 0.8–1.0 ($F < 1.20$) since 1956 within $\pm 20\%$ of the long-term mean; fully recruited F in 1996 was 0.87. Current F was below the threshold reference level of $F_{10\%} = 1.21$, where $F_{\text{REP}} = 1.17$. Yield per recruit (YPR) modeling estimated $F_{0.1} = 0.36$ and $F_{\text{MAX}} = 0.64$. The stock is strictly growth overfished with a 26% reduction in F_{1996} required to maximize (+2% gain) YPR. Longevity was established at 8 y. Tagging studies showed that blue crabs live considerably longer than current convention (3 y). A life-table model reconciled the apparent disparity between this convention and the life-history parameters used in the assessment. At current total mortality (Z) ≈ 1.3 , the mean age of the observed stock would be 1.5 y (153 mm CW [carapace width]); 97.3% of all individuals would be ≤ 3 y of age. The dramatic rise in f since 1945 with the accompanying decline in CPUE was not associated with an increase in F . Catchability (q) has varied with f from 1956 to 1995 as a result of gear saturation. Moderate reductions in f would not result in a proportional reduction in F because of nonconstant q . The blue crab fishery is severely overcapitalized in terms of total effort. As a result of gear saturation, marginal decreases in fishing effort would not realize proportional gains in CPUE, %MSP, or YPR. Substantial economic displacement would be required to maximize YPR. Increases in %MSP and YPR could be realized through increased size limits and regulating the taking of mature female crabs, without the displacement costs associated with effort reductions alone. We recommend a risk-averse management strategy: maintain current F below $F_{10\%}$. Management should be particularly averse to increases in effort that would exacerbate current economic inefficiencies, or to increases in gear efficiency. The latter concern acknowledges the delicate interplay between F and f in the fishery. Management should be proactive: stabilize and enhance the economic viability of the fishery and provide protection for the stock through maintenance of $\geq 10\%$ maximum spawning potential (MSP). It should consider adopting strategies which increase YPR (e.g., size limit measures on both sexes) and spawning potential (e.g., limit directed fisheries on mature female crabs).

KEY WORDS: blue crab, *Callinectes sapidus*, Chesapeake Bay, harvest, population, fishery, exploitation, stock assessment, status, gear saturation

INTRODUCTION

The blue crab (*Callinectes sapidus* Rathbun) supports the most important commercial and recreational fishery in the Chesapeake Bay. The blue crab population of the Chesapeake Bay is distributed throughout the bay and its tidal tributaries. In 1995, the bay-wide commercial landings totaled 33.5×10^6 kg (Rugolo et al., this volume). Recreational fishing on blue crab in the Chesapeake is also important in terms of annual exploitation, however, estimates

of the magnitude of recreational harvest and directed effort are largely unavailable or poorly understood (Knotts 1989). In 1983, 1988, and 1990, the Maryland Department of Natural Resources (MDNR) attempted to estimate recreational blue crab harvest in cooperation with the National Marine Fisheries Service's Marine Recreational Fisheries Statistics Survey. Results of these surveys indicated that the Maryland blue crab recreational harvest represented approximately 78.6, 49.5, and 25.9% (18.6, 9.7, and 5.2×10^6 kg), respectively, of the reported Maryland commercial har-

vest in those years (Stagg et al. 1994). The 1990 survey was regarded as the more rigorous of the three, nevertheless, weaknesses were identified in the definition of the sampling frame and in the estimation of catch rates of certain segments of the fishery (Stagg et al. 1994, Rugolo et al., this volume).

The blue crab supports economically important fisheries nationwide. From 1990–1994, the average United States blue crab landings approximated 96.0×10^6 kg, worth an average \$140.2 million. The ex-vessel value of commercial Chesapeake Bay blue crab harvest in 1990 to 1994 averaged \$53.1 million in comparison. The most recent (1990) recreational harvest expenditures in Maryland were estimated at \$110 million (Stagg et al. 1994, Abbe and Stagg 1996). The Chesapeake Bay has historically represented the area of greatest blue crab production in the United States. From 1990 to 1994, 36.5, 29.2, 28.2, and 6.1% of total United States blue crab landings were derived, respectively, from the Chesapeake Bay, the Gulf of Mexico, the Southern Atlantic, and Middle Atlantic regions.

Chesapeake Bay blue crab landings have demonstrated inherent variability among years (Pearson 1948, Van Engel 1958, Tagatz 1965, Abbe 1983, Lipcius and Van Engel 1990, Abbe and Stagg 1996, Rugolo et al. 1997, Rugolo et al., this volume). Coincident with the recent decline in many important fisheries resources in the Chesapeake, such as oyster, American shad, and striped bass, as well as in several coastal migratory species that historically have supported Bay fisheries (e.g., bluefish, weakfish, summer flounder), more recreational and commercial emphasis has been placed on the Chesapeake Bay blue crab stock. In the mid-1990s, information from Chesapeake Bay fisheries-independent surveys and changing dynamics in the fisheries suggested cause for concern regarding the health of the Bay's blue crab population (Rugolo et al. 1997). Declining commercial yield and research survey abundance indices, decreasing economic performance of commercial fishers, as well as indications of increased removals of females from the stock all stimulated concern among the Bay states about the status of the blue crab stock and the viability of the fisheries (Rugolo et al. 1997, Anon. 1997). This research stemmed from that concern. Our principal aim was to provide a fundamental underpinning for objective decisions on the conservation and utilization of the Chesapeake Bay blue crab population.

In developing this stock assessment, we examined a variety of fisheries-dependent and fisheries-independent time series data that provided information on the status and dynamics of the Chesapeake Bay blue crab stock and its fisheries. Pertinent literature was reviewed for information on blue crab biology, life-history, population dynamics, and the historical performance of the fisheries. Historic and current blue crab research results relevant to the development of the assessment and to the interpretation of its findings were considered. For the purpose of this assessment, the blue crab population of the Chesapeake Bay and its tributaries was considered a unit stock.

This stock assessment is the first integrated analytical approach developed for the blue crab. Although this work provided an efficient use of available data through contemporary fisheries stock assessment approaches, several data and informational deficiencies were identified (Rugolo et al. 1997). We expect that future updates of this assessment will benefit from improved biological and fisheries data, as well as from research results that advance our understanding of vital blue crab life-history and population dynamic characteristics essential to assessing stock status.

We sought the answers to three questions as our operational

framework. First, what is the current status of the stock? Second, what is the optimal, or target level of the stock? Third, what management strategies can be adopted to achieve stock recovery if the stock is suboptimal, or to maintain the stock at current levels should remedial action be unnecessary? An extension of this framework is risk assessment. That is, within the integrated analytical framework of the assessment, a specific aim was to provide decision makers a flexible, adaptive system for considering the risk associated with various management options on long-term stock status and fishery health.

In the development of the *1997 Chesapeake Bay Blue Crab Fishery Management Plan* (Anon. 1997), results of this research were central to decisions regarding the management of this important resource. This research has also contributed to the dialog concerning critical biologic and life-history characteristics of the blue crab and to the understanding of blue crab stock and fishery dynamics. Our work has identified research and information needs critical to an understanding of blue crab population dynamics. This stock assessment was approved in April 1997 by the Chesapeake Bay Stock Assessment Committee (CBSAC) of the National Marine Fisheries Service, NOAA under whose aegis it was conducted and by the member states and participants of the Chesapeake Bay Program.

MATERIALS AND METHODS

Where possible and essential for the reader's understanding, we provide requisite data in tables and figures that were subject to the analysis. Given the scope of this research, we were not able to present all data and information that underlie the assessment. The reader is referred to Rugolo et al. (1997) for a more complete presentation of this information.

Adult Stock

We compiled and examined a variety of extant fishery-dependent and fishery-independent time series data that provided information on the status of the blue crab stock. Indices of relative adult stock abundance were available from several research surveys conducted in the Chesapeake Bay and its tributaries. These included the baywide winter dredge survey (1990 to 1996), the MDNR summer trawl survey (1977 to 1995), the Virginia Institute of Marine Sciences trawl survey (1955 to 1995), the Calvert Cliffs peeler pot survey (1968 to 1995) (Abbe 1983, Abbe and Stagg 1996), the Smith Island scrape survey (1948 to 1972), and the 1987 pot, trotline, and scrape study conducted by the Chesapeake Biological Laboratory, University of Maryland. Using intermediate results of this work, we derived estimates of absolute blue crab stock abundance and biomass for years 1956 to 1995. Trends in relative and absolute abundance were examined to provide a first-order approximation of the current status of the adult stock. Because direct aging of blue crabs is not possible, approximate ages were assigned to survey carapace width (CW) data to derive age-specific indices of abundance as: age 0 (0–59 mm); age 1 (60–119 mm); age 2 (120–79 mm); and female spawners (≥ 130 mm).

Winter Dredge Survey

A Chesapeake Baywide winter dredge survey has been conducted since 1989 through cooperative efforts of the Chesapeake Biological Laboratory and the Virginia Institute of Marine Sciences, College of William and Mary. This survey provides baywide indices of blue crab relative abundance with sampling oc-

curing during the time when crabs aestivate in the sediment. Annual survey sampling consists of a single 100-m tow of a 2.8-m (6 foot) toothed commercial dredge at nearly 1,000 stations throughout the bay. The dredge is equipped with a 15-mm mesh bag, which is effective for the capture of small crabs. Data are collected by sex and size class (mm); catch per unit effort (CPUE) is measured as the number of crabs/1,000 m². Sex-specific age 1+ and age 2+ stock abundance indices are presented in Figures 1 and 2.

Maryland Trawl Survey

The MDNR blue crab trawl survey (1977 to 1995) provides an index of relative abundance for age 0+ crabs measured as the mean number of crabs/tow. Survey sampling has occurred from May to November in the Chester River, the Choptank River, the Potomac River, the Eastern Bay, Tangier Sound, and Pocomoke Sound. In many years, some systems and months were unsampled. To provide an internally consistent measure of CPUE, we selected the subset of the Choptank River, the Eastern Bay, and Pocomoke and Tangier Sounds during July to September, which were sampled fairly consistently each year. Annual indices of abundance were calculated as the mean monthly CPUE for all areas combined. Time series trends in relative abundance for age 1+ male and female crabs are shown in Figure 3.

VIMS Trawl Survey

The Virginia Institute of Marine Sciences (VIMS) has conducted a trawl survey since 1955 to measure trends in relative abundance of selected finfish species. Sampling occurs in the lower Chesapeake Bay and up to the freshwater interface of the James, York, and Rappahannock Rivers. Samples from approximately 100 stations are collected monthly by a 10-m wide shrimp trawl towed for 5 minutes. Catch is sorted and enumerated by species; biological data on a considerable portion (20–50,000/month) of the catch is taken. This trawl program has undergone several modifications in gear and sampling protocol since its inception in 1955. Rugolo et al. (1997) describe the nature of changes in the study that have affected catch rates of key targeted species. Since 1979, the survey sampling protocol has remained essentially unchanged.

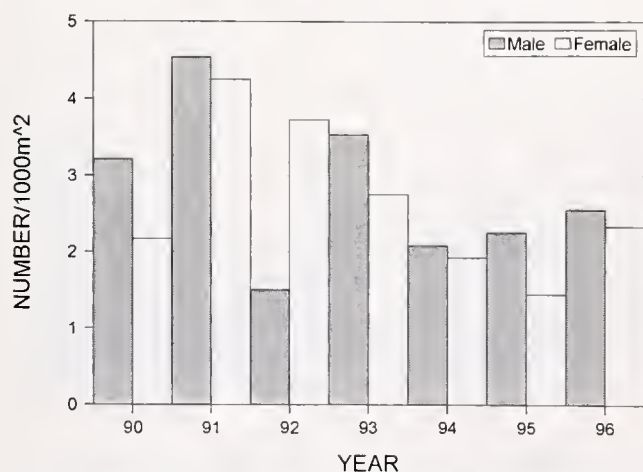


Figure 1. Baywide winter dredge survey relative abundance index (number/1,000 m²) for age 1+ (≥60 mm CW) blue crabs by sex for 1990 to 1996.

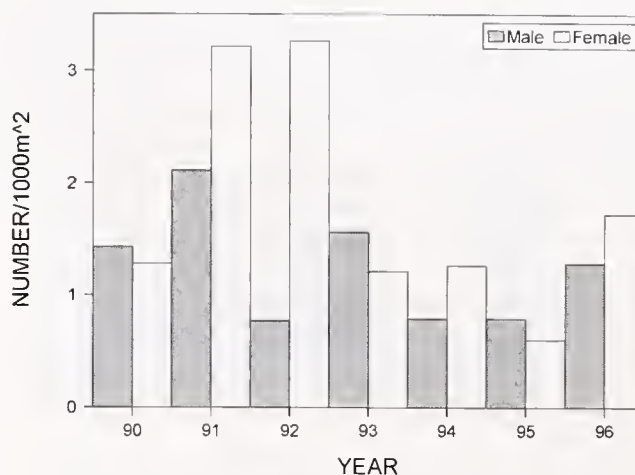


Figure 2. Baywide winter dredge survey relative abundance index (number/1,000 m²) for age 2+ (≥120 mm CW) blue crabs by sex for 1990 to 1996.

Indices of relative abundance for blue crabs are expressed as the geometric mean catch/tow weighted by strata area. Recently, comparative sampling has been conducted by the VIMS survey staff using historical gear configurations in an effort to standardize catch rates associated with each configuration. Provisional corrected blue crab indices of relative 1968 to 1995 abundance were derived based on this analysis and examined for trends in abundance among years. The corrected relative abundance of age 1+ blue crabs collected from the James, York, and Rappahannock Rivers, sexes combined, are shown in Figure 4.

Calvert Cliffs Survey

The Calvert Cliffs pot survey has been conducted since 1968 adjacent to the Calvert Cliffs Nuclear Power Plant in the Chesapeake Bay (Abbe 1983, Abbe 1987, Abbe and Stagg 1996). This survey uses baited commercial peeler pots of 25-mm (1-in) mesh to sample crabs at three sites. The most consistent sampling has occurred between June and November of each year, when up to 60 pots were fished during alternate weeks. From 1968 to 1995, 113,002 blue crabs were caught in 18,106 pot days (Abbe and Stagg 1996). Although peeler pots generally select for crabs larger than 75 mm CW, crabs as small as 38 mm CW are taken.

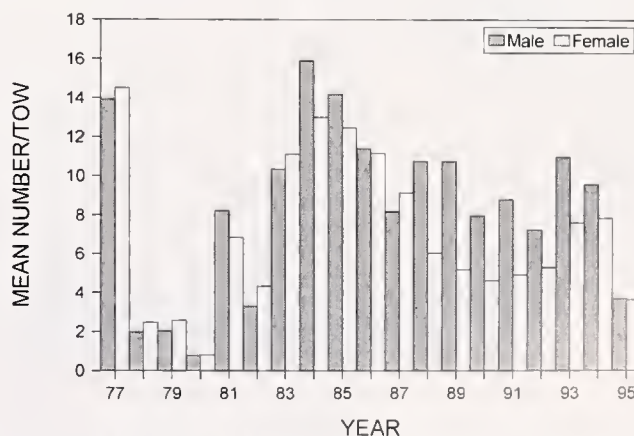


Figure 3. Maryland trawl survey relative abundance index (mean number/tow) for age 1+ (≥60 mm CW) blue crabs by sex for 1977 to 1995.

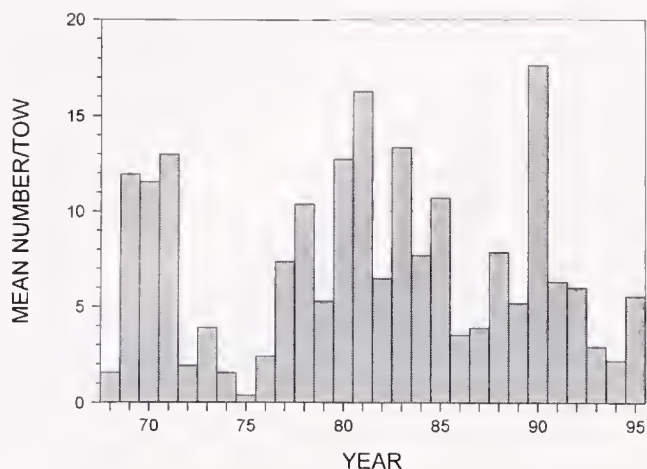


Figure 4. Virginia Institute of Marine Sciences trawl survey corrected relative abundance index (mean number/tow) for age 1+ (≥ 60 mm CW) blue crabs sexes combined for 1968 to 1995.

Approximately 75% of crabs caught in this survey since 1968 were larger than 120 mm CW.

Sex-specific relative abundance from this survey is expressed as the arithmetic mean number/pot. Trends in survey CPUE for age 1+ male and female blue crabs from 1968 to 1996 are shown in Figure 5. The trend in blue crab relative abundance from the Calvert Cliffs survey and the Maryland blue crab harvest are highly correlated (Abbe and Stagg 1996, Rugolo et al. 1997, Rugolo et al., this volume). Correlation coefficients of 0.72 and 0.70 were found between survey CPUE and the Maryland pot and Maryland total harvest, respectively, and 0.88 with the Maryland commercial pot CPUE.

Smith Island Scrape Survey

In a project conducted by the Chesapeake Biological Laboratory, abundance and size composition data on blue crabs were consistently collected from the Smith Island scrape fishery from 1948 to 1972 (except 1955, 1956, and 1959). The two components of the sampling program consisted of weekly mean size and sex composition of crabs captured by the same commercial fisher throughout the season, and the mean daily CPUE (number/fisher/

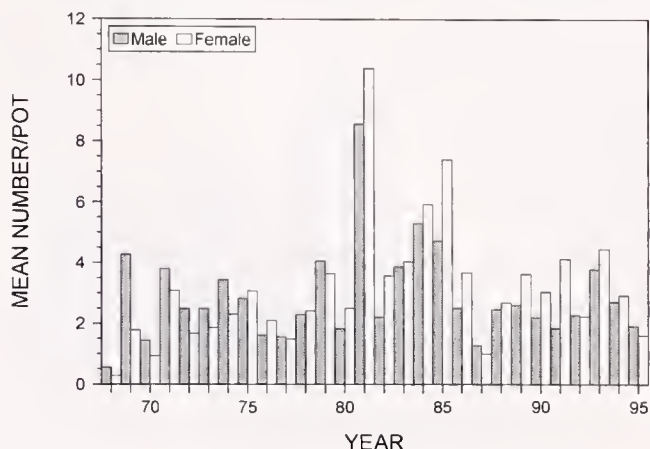


Figure 5. Calvert Cliffs peeler pot survey relative abundance index (mean number/pot) for age 1+ (≥ 60 mm CW) blue crabs sexes by sex for 1968 to 1996.

day) for each week of the season based on a subsample of all Smith Island scrape fishers. The Smith Island survey time series is based on samples drawn from a small geographic area; however, no other survey provides estimates of blue crab abundance over this period. The Smith Island abundance trends provide a unique and useful means to examine historic patterns in Chesapeake Bay blue crab relative abundance.

The scrape fishery likely represents the most unbiased commercial gear for estimation of size structure of the blue crab population ≤ 120 mm CW; larger sizes are captured at lower rates either because of gear selectivity or availability. We considered the nominal age 2+ (≥ 120 mm CW) abundance index to be less reliable than indices for age 0 and age 1 (Figure 6). The Smith Island scrape and Calvert Cliffs pot surveys both provide consistent sampling for blue crabs, and the respective indices of abundance are well correlated in the 5 years (1968 to 1972) when both surveys were conducted (Rugolo et al. 1997). To ascertain the degree of coherence in CPUE between these survey data, log-log regressions were fit to the Smith Island and Calvert Cliffs size class abundance data most efficiently captured by the gears. During 1968 to 1972, the Calvert Cliffs age 1+ and Smith Island age 1 indices were highly correlated ($R^2 = 0.96$) and related according to $CC_{1+} = 8.97^{-4} \times SI_{1+}^{1.41}$. Stronger correlations were found between the CC_1 and SI_1 indices ($R^2 = 0.99$); strong correlations were found between the CC_{1+} and SI_{1+} ($R^2 = 0.95$) and between the CC_{2+} and SI_{1+} indices ($R^2 = 0.93$) (Rugolo et al. 1997).

The fact that these surveys were so highly correlated suggested that a 50 y time series of survey relative abundance could be developed by merging age-specific indices from the two surveys. Because the Calvert Cliffs survey is on-going, we chose to express historic Smith Island relative blue crab abundance in the currency of Calvert Cliffs relative abundance—that is, impute the Calvert Cliffs indices from 1948 to 1972 based on the functional form noted, Calvert Cliffs age 1+ CPUE from 1968 to 1994 (observed) and estimated (1948 to 1972) based on the log-log regression of CC_{1+} on SI_1 CPUE indices are shown in Figure 7. Regression results are shown in the inset.

Fishery-Dependent Stock Measures

Estimates of the total population abundance of the peeler/soft crab component of the stock and the hard crab component of the

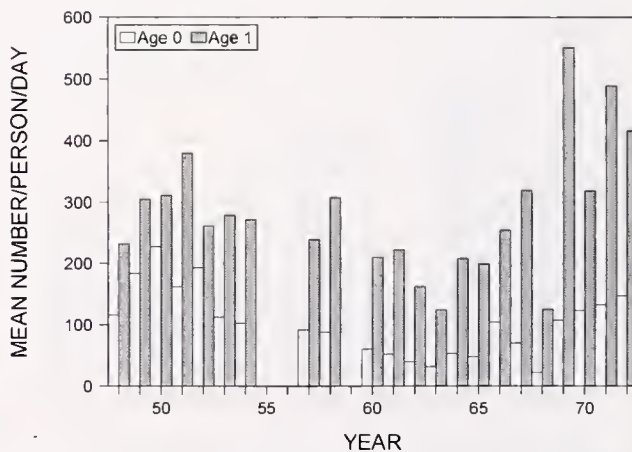


Figure 6. Smith Island scrape survey relative abundance index (mean number/person/day) for age 0 (0–59 mm CW) and age 1 (60–119 mm CW) blue crabs sexes combined for 1948 to 1972 (except 1955, 1956, and 1959).

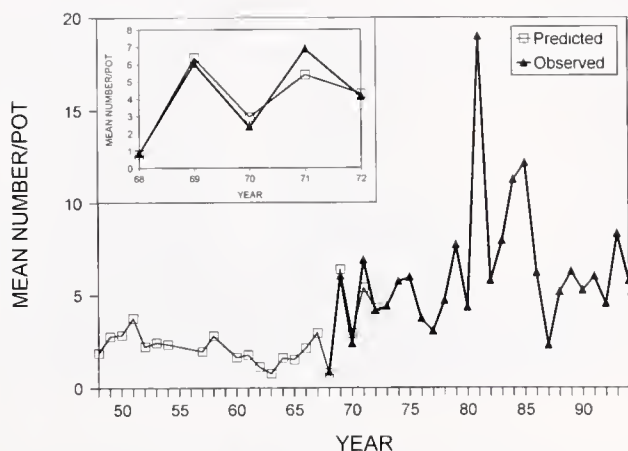


Figure 7. Calvert Cliffs peeler pot survey age 1+ (≥ 60 mm CW) relative abundance index showing observed (1968 to 1994) and estimated (1948 to 1972) abundance indices based on log-log regression of Calvert Cliffs age 1+ abundance on Smith Island age 1 abundance. Regression results are shown in inset.

stock were made for 1956 to 1995. These population estimates were derived using historic hard and peeler/soft crab harvest data and estimated annual exploitation rate (μ) (Rugolo et al. 1997). Under this formulation, the annual exploitation rate in year t [μ_t] is:

$$\mu_t = [F_t A/Z] = [F_t^* (1 - e^{-(M + F_t)})]/(M + F_t); \quad (1)$$

where F = fishing mortality, Z = total mortality, and M = natural mortality. These calculations assume Type II fisheries with $M = 0.375$, and the partial recruitment of age 1 crabs (PR_1) equal to 0.75, and $PR_{2+} = 1.0$ for age 2+ crabs. The fishing mortality rate term in Eq. 1 is multiplied by the PR scaler for each stock component—that is, by PR_1 for peeler/soft crabs and PR_{2+} for hard crabs. Total absolute abundance (N_t) in year t is derived by rearrangement of the catch equation (Baranov 1918):

$$N_t = C_t/\mu_t \quad (2)$$

Historical commercial hard and peeler/soft crab harvest (C_t) data were available for Maryland, Virginia, and baywide fisheries (Rugolo et al., this volume). Based on previous estimates of the recreational harvest of blue crabs (Stagg et al. 1994), the reported commercial harvest in each year was expanded by 25% to account for expected removals from the stock caused by recreational fishing. The resulting population estimates can be considered underestimates as a result of inadequate or under-reporting of commercial harvest and the severe underestimation of peeler/soft crab landings (Rugolo et al., this volume). Estimates of the total population abundance for the peeler/soft crab and the hard crab stock components were derived using annual exploitation rates (μ_t) based on the Calvert Cliffs and VIMS trawl survey length-based estimates of F (Table 1). The time series trends in estimated total abundance of the peeler/soft crab and hard crab stocks for 1956 to 1995 are shown in Figure 8.

Juvenile Recruitment

Current and historic juvenile recruitment was assessed by examining fishery-independent age 0 and age 1 relative abundance indices from the winter dredge survey, Calvert Cliffs pot survey, VIMS trawl survey, MDNR trawl survey, and the Smith Island

scrape survey. Estimated absolute baywide peeler/soft crab abundance was also examined. The trend in age 0 relative abundance measured by the baywide winter dredge survey in 1990 to 1995 is shown in Figure 9. Relative abundance of male and female age 0 blue crabs measured by the MDNR trawl survey for years 1977 to 1995 is shown in Figure 10. Figure 11 presents the time series of corrected relative age 0 abundance for years 1968 to 1995, sexes combined, from the VIMS trawl survey. Commercial peeler pots employed in the Calvert Cliffs survey do not adequately select the age 0 group (0–59 mm CW). Calvert Cliffs survey abundance

TABLE 1.

Estimates of total absolute abundance (N) (10^6) of crabs based on reported harvest and survey-based estimates of annual exploitation rate on the peeler/soft and hard crab components of the Chesapeake Bay stock for 1956 to 1995, and mean abundance with standard error for the respective periods.

Year	Calvert Cliffs-Based N			VIMS Trawl-Based N		
	P/Soft	Hard	Total	P/Soft	Hard	Total
1956	—	—	—	40.3	245.6	285.9
1957	—	—	—	51.3	255.0	306.4
1958	—	—	—	46.9	222.0	268.9
1959	—	—	—	32.2	204.6	236.7
1960	—	—	—	44.9	327.8	372.7
1961	—	—	—	43.4	343.2	386.6
1962	—	—	—	53.8	389.4	443.2
1963	—	—	—	31.4	303.2	334.6
1964	—	—	—	46.3	355.9	402.2
1965	—	—	—	38.1	398.0	436.1
1966	—	—	—	30.6	512.0	542.6
1967	—	—	—	36.3	409.4	445.7
1968	30.9	420.0	450.8	19.5	276.0	295.5
1969	43.8	288.6	332.4	45.8	300.1	345.8
1970	29.3	380.4	409.7	29.6	383.6	413.2
1971	22.9	376.9	399.8	21.8	360.8	382.5
1972	26.0	379.1	405.1	24.0	353.8	377.8
1973	32.1	348.4	380.4	21.8	246.8	268.7
1974	34.2	387.5	421.7	26.7	309.7	336.4
1975	29.8	344.8	374.6	22.4	266.6	288.9
1976	27.4	272.9	300.4	25.4	254.4	279.8
1977	29.5	417.3	446.9	23.0	331.1	354.1
1978	22.4	331.0	353.4	19.4	290.6	310.1
1979	130.6	373.4	504.0	123.2	354.3	477.5
1980	25.5	418.6	444.0	23.3	385.3	408.6
1981	30.6	545.2	575.8	32.8	580.6	613.4
1982	48.7	610.9	659.6	42.5	538.9	581.3
1983	60.7	618.0	678.7	54.8	563.4	618.2
1984	30.2	491.8	522.0	28.1	462.6	490.8
1985	40.8	490.2	531.0	37.3	452.9	490.3
1986	24.2	462.0	486.2	25.5	486.1	511.6
1987	30.1	474.0	504.1	28.2	446.5	474.6
1988	27.9	484.5	512.4	26.5	461.6	488.1
1989	30.7	496.8	527.5	31.7	510.6	542.3
1990	30.5	554.1	584.6	29.3	534.3	563.6
1991	38.0	532.0	569.9	41.3	573.7	614.9
1992	17.8	277.9	295.8	22.1	337.6	359.8
1993	39.7	582.4	622.2	41.6	607.1	648.8
1994	29.8	370.1	399.9	29.8	370.1	399.9
1995	35.5	365.3	400.8	39.2	399.0	438.1
Mean	35.7	431.9	467.6	35.8	385.1	420.9
SE	3.9	18.6	19.5	2.7	17.3	17.6

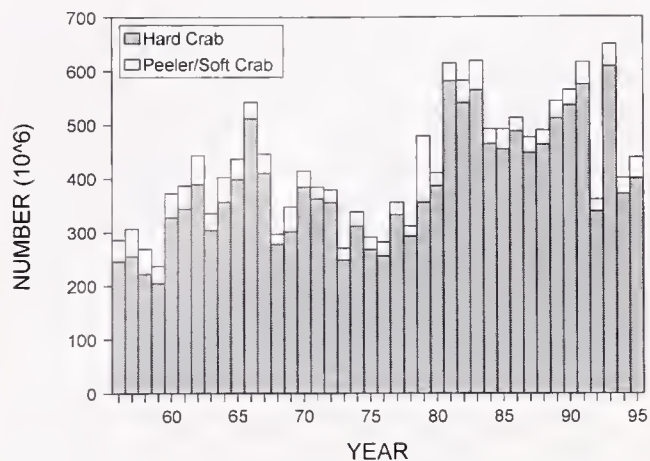


Figure 8. Estimated Chesapeake Baywide total absolute abundance of crabs in the peeler/soft crab and hard crab components of the stock based on reported harvest values and the VIMS survey-based estimates of annual exploitation rate for 1956 to 1995.

indices for age 1 (60–119 mm CW) crabs were derived for 1968 to 1995 by sex (Fig. 12). Trends in age 0 blue crab relative abundance for years 1948 to 1972 measured by the Smith Island survey are shown in Figure 6, absent years 1955, 1956, and 1959.

Fishery Yield and Catch per Unit Effort

Historical (1945 to 1995) Maryland and Virginia commercial landings of hard and peeler/soft crabs and total nominal directed effort were compiled (Table 2) (Rugolo et al., this volume). Data from 1945 to 1972 for Virginia and through 1980 for Maryland were obtained from the Bureau of Commercial Fisheries (later the National Marine Fisheries Service) annual volumes of the *Fisheries Statistics of the United States*. Data in subsequent years were provided by the Virginia Marine Resources Commission (VMRC) and MDNR. These commercial fisheries data are unadjusted reported landings. Historic effort data were most complete for the baywide pot fishery, accounting for 40–80% of the landings from all jurisdictions from 1945 to 1995, and for 70% since 1981 (Rugolo et al., this volume). Directed fishing effort from the other

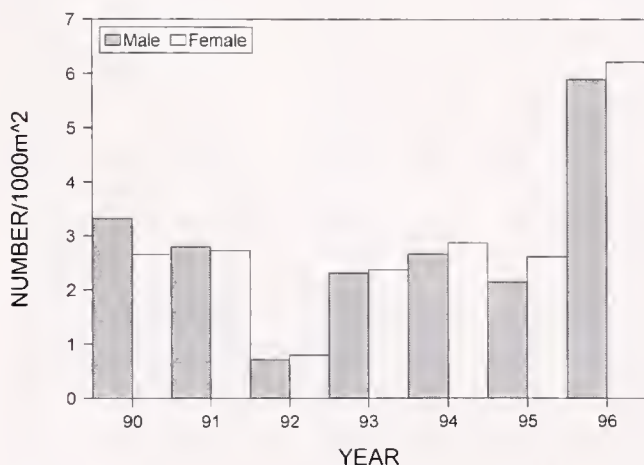


Figure 9. Baywide winter dredge survey relative abundance index (number/1,000 m²) for age 0 (0–59 mm CW) blue crabs by sex for 1990 to 1996.

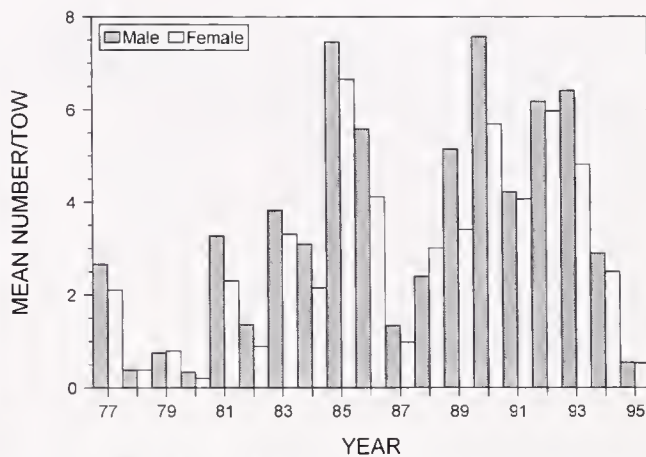


Figure 10. Maryland trawl survey relative abundance index (mean number/tow) for age 0 (0–59 mm CW) blue crabs by sex for 1977 to 1995.

two principal commercial gear types (trotline and dredge) were converted to equivalent pot units and combined to provide a baywide measure of total nominal effort for 1945 to 1995 (Fig. 13; Rugolo et al., this volume). These annual effort data were consistently derived and judged to provide a suitable basis for interannual comparisons of the trend in effort during the last five decades.

Total baywide hard plus peeler/soft crab harvest (Fig. 14) were divided by total nominal effort to provide a measure of CPUE in the baywide fisheries for years 1945 to 1995 (Table 2, Fig. 15). Based on recent time series of harvest and effort from 1982 to 1995, trends in relative stock abundance from the Maryland pot and trotline fisheries were derived (Fig. 16). Commercial harvest and effort data from the Potomac River were provided by the Potomac River Fisheries Commission (PRFC) for years 1964 to 1996 (Table 3, Fig. 17).

Surplus Production Analysis

Surplus production modeling was developed for the purpose of estimating maximum sustainable yield (MSY) for the combined Chesapeake Bay commercial fisheries. We developed production

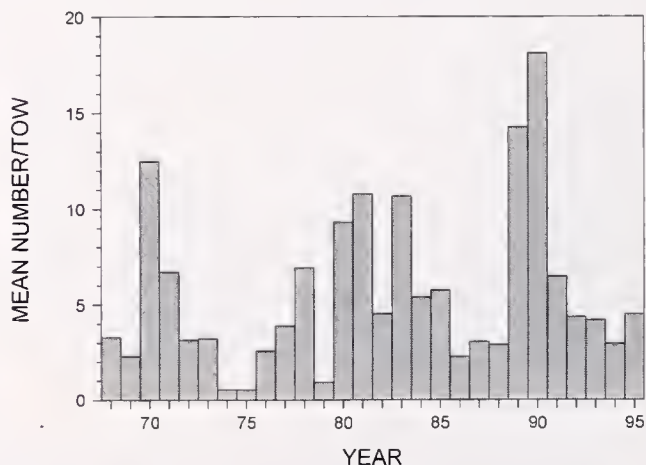


Figure 11. Virginia Institute of Marine Sciences trawl survey corrected relative abundance index (mean number/tow) for age 0 (0–59 mm CW) blue crabs, sexes combined, for 1968 to 1995.

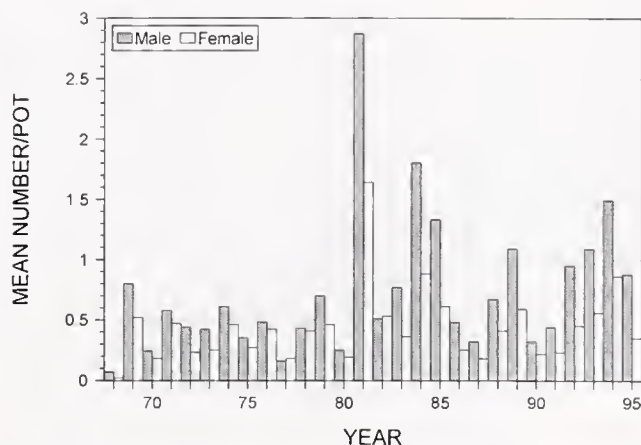


Figure 12. Calvert Cliffs peeler pot survey relative abundance index (mean number/pot) for age 1 (60–119 mm CW) blue crabs by sex for 1968 to 1996.

models of Schaefer (1954) and Fox (1970) using baywide commercial harvest and nominal directed effort for 1945 to 1995 (Table 2). This analysis updated that of Tang (1983), who used harvest and effort data through 1981. The resulting fits of the Schaefer and Fox production models are shown in Figures 18 and 19.

Stock–Recruitment

We explored blue crab stock–recruitment (S–R) modeling using a variety of spawning stock and recruitment abundance indices. The purpose of these efforts was twofold: (1) to determine if a consistent, representative time series of spawning stock and recruitment measures was available to characterize the relationship between spawning stock and subsequent recruitment; and (2) to examine whether the relationship between stock–recruitment could be improved by incorporating density-independent factors that influence juvenile survivorship or recruitment to the stock. We fit the Ricker (1954) stock–recruitment model to a total of six spawning stock and seven recruit metrics for a total of 42 pairwise combinations of S–R relationships. We refer the reader to Rugolo et al. (1997) for a more thorough presentation of this analysis. Results of the two best model fits between the VIMS trawl survey female spawning stock (≥ 130 mm CW) index in year t versus the VIMS and MDNR trawl survey age 0 indices in year $t+1$ are shown in Figures 20 and 21, respectively.

Preliminary analysis using the best S–R model fits were conducted using a method described in Tang (1985) to examine if environmental factors assumed to affect juvenile blue crab survivorship and recruitment to the stock could improve the stock–recruitment relationship. We examined bottom measures of temperature, salinity, and dissolved oxygen in the mainstem bay and freshwater inflow rates measured as cumulative river discharge from the Susquehanna River through the mouth of the Chesapeake Bay. Results of this analysis were indeterminate, because these environmental factors were not found to correlate significantly with the density-independent parameter of the Ricker (1954) model. Further analysis of density-independent effects on recruitment success in blue crabs is in development using more determinative environmental measures (Rugolo et al. 1997).

Exploitation Rates

To gauge the current and historic performance of the Chesapeake Bay blue crab fisheries, we derived measures of relative

exploitation rate (1968 to 1995) and absolute exploitation rate (1956 to 1995) on the hard and peeler/soft crab components of the stock. Measures of relative exploitation rate provided a basis for assessing the time series trends in exploitation rate and for detecting a rise in exploitation as recent anecdotal information suggested. Estimates of absolute exploitation rates represent the fraction of the stock removed by fishing.

Relative Exploitation Rates

Relative exploitation rate was calculated using an estimate of the fraction of the stock removed by the fisheries. From the Baranov (1918) catch equation ($C = F \cdot \bar{B}$) we see that fishing mortality rate (F) is proportional to catch (C) divided by average biomass (\bar{B}). If an adequate index of stock biomass (\bar{B}) can be determined from survey or fishery CPUE, then relative exploitation rate (μ_{REL}) can be calculated as the ratio of the reported catch biomass (C) to the index of stock biomass (CPUE):

$$\mu_{REL} = C/CPUE \quad (3)$$

From this concept we derived relative exploitation rate (μ_{REL}) in year t as:

$$\mu_{REL} = \frac{\text{Catch}_t \text{ (kg)}}{\text{Survey Abundance Index}_t \text{ (kg)}} \quad (4)$$

Relative exploitation rates were derived using baywide total commercial harvest (Table 2, Fig. 14) and two fishery-independent measures of relative exploitable stock biomass: the Calvert Cliffs pot survey and the VIMS trawl survey CPUE indices. Figure 22 illustrates the time series trend in μ_{REL} on the baywide stock using Calvert Cliffs age 1+ and age 2+ stock biomass indices for 1968 to 1995. Chesapeake Bay relative exploitation rates during 1968 to 1995 were also computed using the VIMS age 1+ and age 2+ stock biomass measures (Fig. 23). A time series trend in μ_{REL} for the Maryland blue crab fishery was derived using the Calvert Cliffs age 1+ and age 2+ abundance indices and the Maryland blue crab harvest in years 1968 to 1995 (Fig. 24).

Absolute Exploitation Rate

The rates of annual exploitation on the hard crab and peeler/soft crab components of the stock were estimated for 1956 to 1995 using the length-based estimates of instantaneous total mortality rate (Z), the partial recruitment of each stock component to fully recruited F , and the instantaneous rate of natural mortality (M). Length-based rates of Z were derived using the Calvert Cliffs and VIMS survey carapace width frequency data (see Total and Fishing Mortality Rates section, below). For these calculations, we assumed Type II fisheries with $M = 0.375$, peeler/soft crab $PR_0 = 0.75$ and hard crab $PR_{1+} = 1.0$. In this formulation, absolute exploitation rate μ_{ABS} in year t was derived as:

$$\mu_{ABS} = \frac{F_t A}{Z} = \frac{F_t (1 - e^{-(M+F_t)})}{(M + F_t)} \quad (5)$$

Width-based estimates of instantaneous fishing mortality rate (F), computed from the VIMS and Calvert Cliffs survey data, as well as derived annual exploitation rates (μ_{ABS}) on the peeler/soft and hard crab stock components are shown in Table 4 and Figures 25 and 26.

TABLE 2.

Commerical harvest (10^3 kg) of hard and soft blue crab from Maryland and Virginia and the baywide total harvest, nominal effort,^a and standard catch per unit effort from 1945 to 1995.

Year	Maryland			Virginia			Total Harvest	Baywide-Nominal Effort	Std. CPUE
	Hard	Soft	Total	Hard	Soft	Total			
1945	8,172	769	8,941	7,894	815	8,709	17,650	89,601	0.434
1946	11,264	1,169	12,433	11,268	731	11,999	24,432	120,031	0.449
1947	11,312	1,361	12,674	14,401	1,087	15,488	28,162	100,256	0.619
1948	9,123	842	9,965	17,907	1,232	19,140	29,105	128,027	0.501
1949	9,792	1,054	10,847	17,052	1,117	18,168	29,015	141,889	0.451
1950	12,177	1,311	13,488	19,461	1,437	20,898	34,385	164,171	0.462
1951	12,024	914	12,939	15,763	1,778	17,542	30,480	165,932	0.405
1952	11,760	712	12,473	14,054	986	15,039	27,512	137,185	0.442
1953	11,958	864	12,823	13,702	1,162	14,864	27,687	159,316	0.383
1954	8,151	501	8,652	13,671	934	14,605	23,258	149,313	0.343
1955	6,908	545	7,453	11,432	804	12,236	19,688	165,734	0.262
1956	9,618	829	10,448	10,423	786	11,209	21,656	169,062	0.282
1957	12,419	1,554	13,973	10,054	716	10,769	24,742	203,852	0.268
1958	11,571	1,439	13,010	7,227	562	7,790	20,800	198,439	0.231
1959	9,405	890	10,295	8,100	483	8,584	18,879	260,846	0.160
1960	12,044	1,263	13,307	16,051	624	16,675	29,982	298,412	0.222
1961	12,002	1,220	13,222	17,711	619	18,330	31,552	264,643	0.263
1962	12,447	1,763	14,210	21,909	549	22,458	36,668	291,079	0.278
1963	7,588	955	8,543	19,102	376	19,478	28,021	326,158	0.189
1964	10,112	1,584	11,696	20,825	363	21,188	32,884	277,271	0.262
1965	14,170	1,181	15,351	20,257	432	20,689	36,040	302,071	0.263
1966	16,429	854	17,283	28,112	442	28,554	45,837	373,684	0.270
1967	11,057	987	12,044	24,863	552	25,415	37,459	340,013	0.243
1968	4,184	454	4,639	20,336	366	20,702	25,340	326,144	0.171
1969	10,356	1,015	11,370	15,281	894	16,175	27,545	375,656	0.162
1970	11,164	715	11,878	18,383	380	18,763	30,641	400,711	0.169
1971	11,697	677	12,374	21,153	292	21,445	33,819	331,511	0.225
1972	10,450	692	11,142	20,883	340	21,222	32,365	334,223	0.214
1973	8,700	667	9,366	15,520	391	15,912	25,278	308,727	0.181
1974	10,364	815	11,179	17,071	339	17,410	28,589	300,465	0.210
1975	10,549	708	11,256	14,186	321	14,507	25,764	323,645	0.176
1976	8,496	627	9,123	11,469	339	11,808	20,931	372,001	0.124
1977	9,058	525	9,583	16,063	310	16,372	25,955	391,517	0.146
1978	7,272	389	7,661	15,887	351	16,238	23,899	461,145	0.114
1979	10,771	4,157	14,928	16,939	489	17,429	32,357	548,744	0.130
1980	10,823	489	11,312	15,189	261	15,450	26,762	490,179	0.120
1981	25,761	977	26,738	20,798	327	21,125	47,863	749,997	0.141
1982	20,814	1,221	22,035	21,785	424	22,209	44,244	667,203	0.146
1983	23,740	1,804	25,543	21,259	356	21,615	47,158	571,223	0.182
1984	19,917	878	20,794	22,273	400	22,672	43,466	536,019	0.179
1985	23,923	1,259	25,182	17,234	458	17,692	42,873	498,308	0.190
1986	20,379	670	21,049	15,467	257	15,724	36,773	545,847	0.149
1987	18,530	811	19,341	13,453	180	13,633	32,974	526,384	0.138
1988	18,397	502	18,899	15,116	441	15,556	34,456	479,227	0.159
1989	18,652	555	19,207	18,297	561	18,858	38,065	535,291	0.157
1990	19,809	642	20,451	20,350	431	20,781	41,232	439,217	0.207
1991	20,748	789	21,537	19,082	604	19,686	41,223	511,988	0.178
1992	13,449	546	13,994	10,571	226	10,797	24,791	460,488	0.119
1993	25,137	837	25,974	24,596	846	25,442	51,416	612,916	0.185
1994	18,998	746	19,745	15,502	658	16,160	35,904	494,597	0.160
1995	18,358	772	19,130	13,542	785	14,327	33,457	614,767	0.120

^a Nominal effort standardized to pot effort.

Total and Fishing Mortality Rates

Contemporary fisheries stock assessment relies on a variety of analytical approaches to estimate fishing mortality rate (F). Where reliable age data exist, catch-at-age analysis, such as virtual popu-

lation analysis or other age-based methods, are often employed. Where age data are unavailable, and suitable measures of the length-frequency of the catch exist, catch-at-size analysis can be employed, given specification of the growth model of the target organism. Blue crabs present challenges in estimating F, because

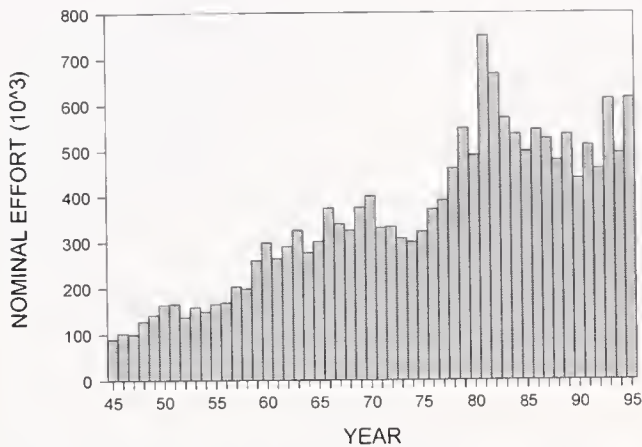


Figure 13. Chesapeake Baywide blue crab nominal fishing effort (10^3 pots) for 1945 to 1995.

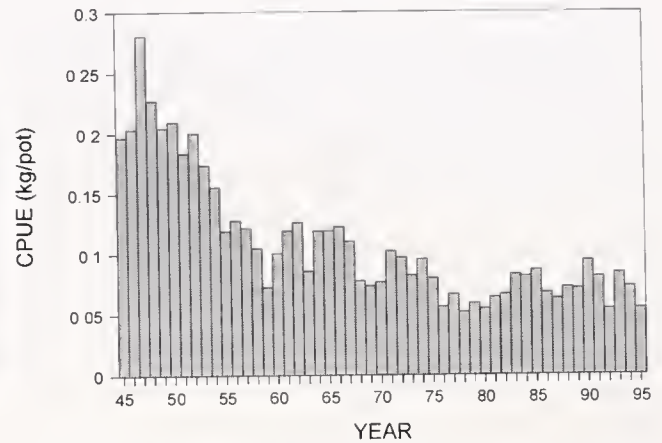


Figure 15. Chesapeake Baywide blue crab commercial CPUE (kg/pot) for 1945 to 1995.

both size and age composition of the landings, as well as the age structure of fishery-independent survey data are unavailable. A reliable method of age determination in blue crabs is not available. The absence of these data precluded many standard analytical stock assessment approaches to estimate F on the Chesapeake Bay blue crab stock.

In instances where representative annual measures of the length frequency (width frequency for crabs) of the stock are available, as well as the growth in length with age, length-based approaches can provide reliable estimates of many population dynamic and fishery parameters. Beverton and Holt (1957) developed an approach for estimating instantaneous total mortality Z from the mean length (\bar{L}) of the catch and the von Bertalanffy (1938) model parameters K and L_∞ . Hoenig (1987) suggested that the Beverton and Holt formulation is biased in the estimation of Z when \bar{L} approaches the length at full recruitment to the gear (L^1). We selected the Hoenig refinement to estimate Z on the Chesapeake Bay blue crab population, because we considered that this bias was operative for available data:

$$Z = \log_e \frac{[e^{-K(\bar{CW} - CW_\infty)}] + CW_\infty - CW^1}{(\bar{CW} - CW^1)} \quad (6)$$

where, K and CW_∞ = curvature and maximum carapace width parameters from the von Bertalanffy model; \bar{CW} = mean total

CW (mm) of crabs of length CW^1 and longer; and, CW^1 = carapace width (mm) at full recruitment to the gear.

Carapace width frequency data used in this analysis were obtained from five fishery-independent surveys, the VIMS and MDNR trawl surveys, the baywide winter dredge survey, the Calvert Cliffs pot survey, and the Chesapeake Biological Laboratory pot, scrape, and trotline study.

Carapace width frequency distributions were prepared for sexes combined in 10 mm width groups, because examination of sex-specific width distributions displayed no pronounced within year differences (Rugolo et al. 1997). Based on resulting width frequencies, gear selection characteristics, survey location, and related life-history information, full recruitment to the survey gear (CW^1) was set at 120 mm. Accordingly, the 120–29 mm and larger width groups corresponding to age 2+ crabs were fully recruited to F . The only exception was the 1987 CBL study using scrape gear, for which full recruitment was set at 60 mm carapace width. In this instance, resulting total mortality estimates included partially recruited age 1 crabs.

Width-based estimates of Z for sexes combined using MDNR and VIMS trawl survey data are shown in Figure 27. Corresponding estimates based on the baywide winter dredge survey, Calvert Cliffs pot survey, and 1987 CBL study are shown in Figure 28.

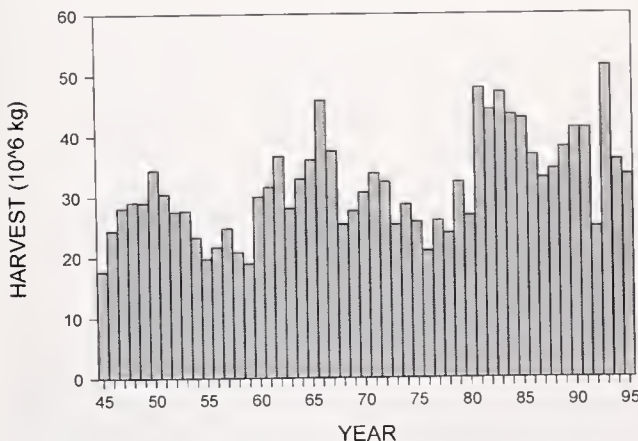


Figure 14. Chesapeake Baywide reported commercial blue crab harvest (10^6 kg) for 1945 to 1995.

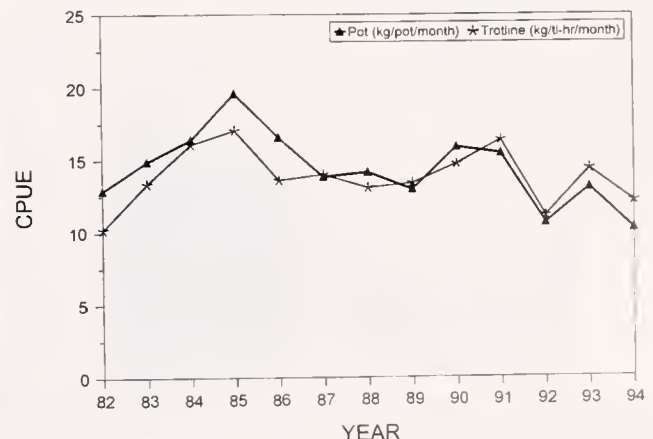


Figure 16. Maryland CPUE for the commercial pot (mean kg/pot/month) and trotline (mean kg/trotline-hr/month) fisheries for 1982 to 1994.

TABLE 3.

Potomac River blue crab harvest (10^3 kg) and effort (number of commercial blue crab licenses issued) for 1964 to 1996.

Year	Harvest	Effort
1964	1,301	226
1965	1,490	231
1966	1,589	256
1967	1,111	244
1968	689	223
1969	736	234
1970	864	224
1971	863	226
1972	963	228
1973	727	247
1974	757	273
1975	943	301
1976	902	338
1977	357	384
1978	1,007	395
1979	1,323	349
1980	n/a	336
1981	n/a	375
1982	1,893	414
1983	2,221	397
1984	1,802	418
1985	2,734	400
1986	2,657	404
1987	2,173	387
1988	2,251	366
1989	2,411	363
1990	2,321	386
1991	3,255	390
1992	2,620	419
1993	3,400	459
1994	2,696	442
1995	1,823	500
1996	2,508	570

Annual fishing mortality rates (F) were obtained from these survey-based estimates of Z by subtraction of $M = 0.375$ (Table 4). The reader is referred to Rugolo et al. (1997) for a more complete presentation of data tables and representative width frequency histograms from these surveys.

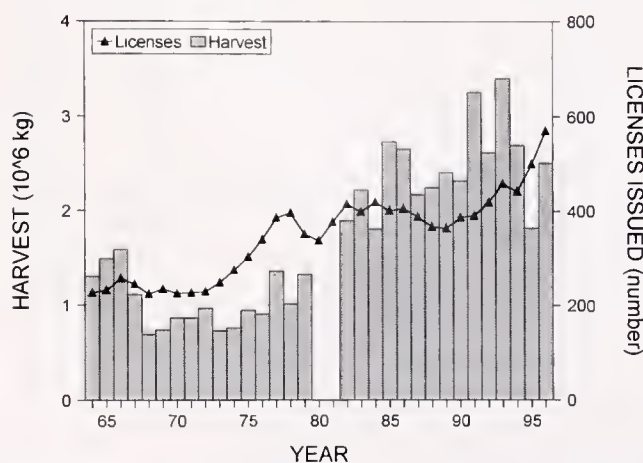


Figure 17. Potomac River blue crab commercial harvest (10^6 kg) and number of commercial blue crab licenses issued from 1964 to 1996.

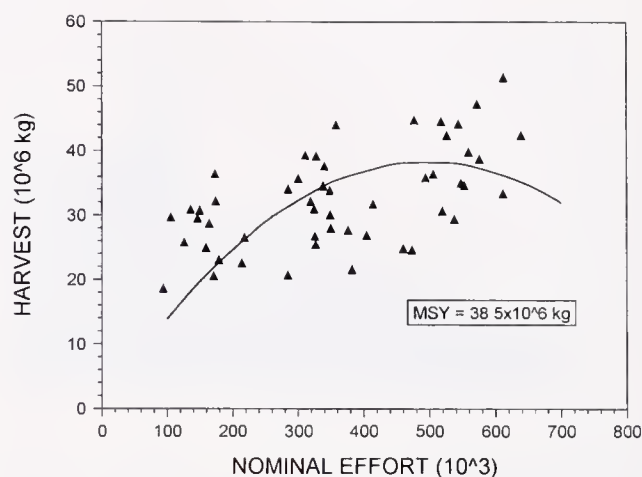


Figure 18. Schaefer surplus production model for baywide blue crab stock based on commercial harvest and nominal effort for 1945 to 1995. Estimated MSY is for commercial fishery only.

Life History Characteristics

Longevity

One of the population dynamic characteristics fundamental to stock assessment is longevity. Longevity is the expected maximum age that the species would attain under conditions of no fishing (i.e., $F = 0$)—the so-called virgin stock. For practical considerations, longevity can be considered as the age which the upper 95th percentile of the unexploited population attains. The current convention is that blue crabs in the Chesapeake Bay live a maximum of 3 y. even in the absence of fishing (Van Engel 1958). This convention is based on the observation of blue crabs held in captivity.

Results of a tagging study conducted in Albemarle Sound, North Carolina provided compelling evidence that blue crabs attain an age of at least 5 years (Fishler 1965). A life span of 5 y is likely a minimum estimate of blue crab longevity, because intense commercial fishing on the stock was coincident with the study; thus, violating the assumption of virgin conditions. Based on Fishler

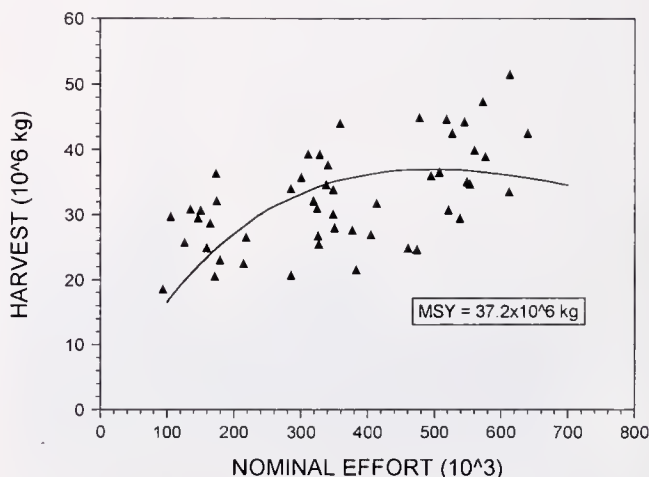


Figure 19. Fox surplus production model for baywide blue crab stock based on commercial harvest and nominal effort for 1945 to 1995. Estimated MSY is for commercial fishery only.

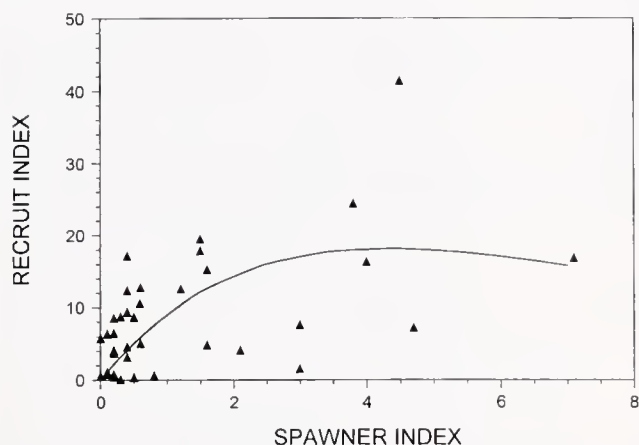


Figure 20. Ricker stock–recruitment model based on the VIMS trawl survey female spawning stock index (≥ 130 mm CW) and age 0 (0–59 mm CW) recruitment index for years 1956 to 1995.

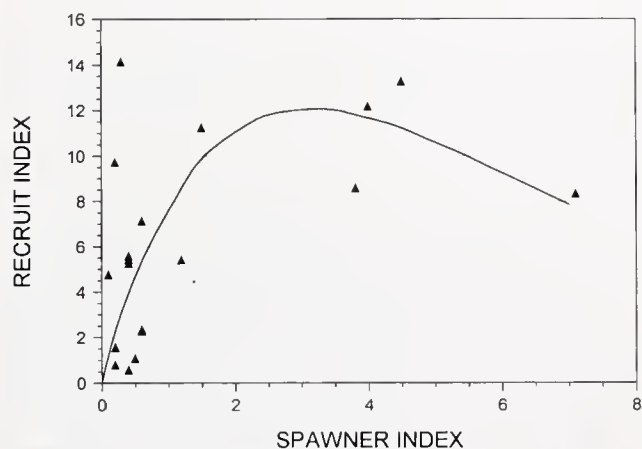


Figure 21. Ricker stock–recruitment model based on the VIMS trawl survey female spawning stock index (≥ 130 mm CW) and the MDNR trawl survey age 0 recruitment index for years 1977 to 1995.

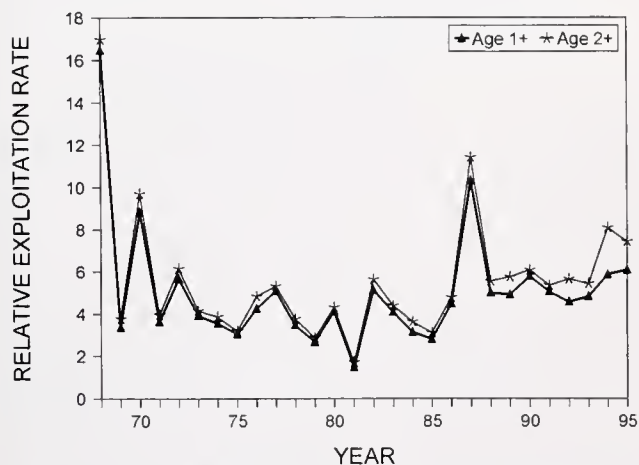


Figure 22. Relative exploitation rate on the blue crab stock calculated from baywide harvest and the Calvert Cliffs pot survey abundance indices (kg/pot) for age 1+ (≥ 60 mm CW) and age 2+ (≥ 120 mm CW) blue crabs.

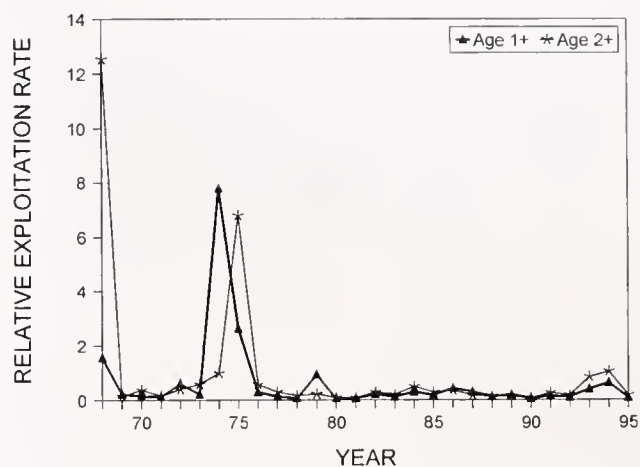


Figure 23. Relative exploitation rate on the blue crab stock calculated from baywide harvest and the VIMS trawl survey abundance indices (kg/tow) for age 1+ (≥ 60 mm CW) and age 2+ (≥ 120 mm CW) blue crabs.

(1965), we initially assumed that longevity in blue crabs was 6 years. Results of a 1989 tagging study (McConnaugha 1991) on mature female blue crabs in the Chesapeake Bay corroborated Fishler's findings of age 5 y blue crabs present in the stock; additional tag returns indicated that 6-year-old crabs were also present. In 1995, two tags were recovered from the 1989 releases, one each in the spring and fall of 1995. Because these two recoveries were mature females (1.5–2 y) when released, this suggested that female blue crabs between 7.5–8 years of age exist in the Chesapeake Bay stock under moderate-to-full rates of fishing mortality. Given this information, we established a maximum theoretical age (longevity) of 8 y for the Chesapeake Bay blue crab.

The use of an older maximum age from the range of likely values is risk averse, because natural mortality (M) becomes a smaller component of Z , and the threshold fishing mortality rates are lowered. Rugolo et al. (1997) examined the impacts on estimated mortality (Z) and biological reference points associated with longevity of 4 y and 6 y. Results of their analysis illustrated that the

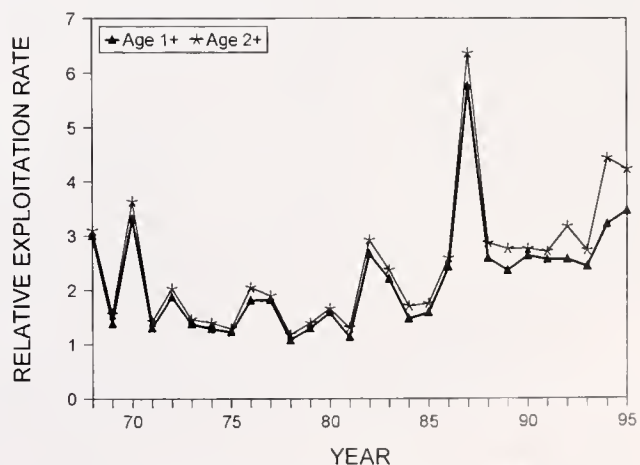


Figure 24. Relative exploitation rate on the blue crab stock calculated from Maryland harvest and the Calvert Cliffs pot survey abundance indices (kg/pot) for age 1+ (≥ 60 mm CW) and age 2+ (≥ 120 mm CW) blue crabs.

TABLE 4.

Length-based estimates of instantaneous fishing mortality rate (F) and exploitation rate (μ) on the peeler/soft and hard crab components of the Chesapeake Bay stock using Calvert Cliffs (CC) pot survey (1968 to 1995) and VIMS trawl survey (1956 to 1995) carapace-width frequency data and mean exploitation rate with standard error for the respective periods.

Year	Exploitation Rate					
	(F)		P/Soft		Hard	
	CC	VIMS	CC	VIMS	CC	VIMS
1956		0.88		0.41		0.50
1957		1.01		0.45		0.55
1958		0.96		0.44		0.53
1959		0.96		0.44		0.53
1960		0.94		0.43		0.53
1961		0.95		0.43		0.53
1962		0.97		0.44		0.53
1963		0.95		0.43		0.53
1964		0.94		0.43		0.52
1965		0.95		0.43		0.53
1966		0.95		0.43		0.53
1967		0.95		0.43		0.53
1968	0.52	0.94	0.27	0.43	0.34	0.52
1969	0.99	0.93	0.45	0.43	0.54	0.52
1970	0.80	0.79	0.38	0.38	0.47	0.47
1971	0.95	1.02	0.43	0.46	0.53	0.55
1972	0.87	0.97	0.41	0.44	0.50	0.53
1973	0.68	1.16	0.34	0.50	0.42	0.59
1974	0.70	0.98	0.35	0.44	0.43	0.54
1975	0.72	1.07	0.35	0.47	0.44	0.57
1976	0.74	0.82	0.36	0.39	0.45	0.48
1977	0.56	0.77	0.29	0.37	0.36	0.46
1978	0.68	0.82	0.34	0.39	0.42	0.48
1979	0.75	0.81	0.37	0.39	0.45	0.47
1980	0.59	0.66	0.30	0.33	0.38	0.41
1981	0.96	0.87	0.44	0.41	0.53	0.50
1982	0.70	0.84	0.35	0.40	0.43	0.49
1983	0.75	0.86	0.37	0.40	0.45	0.49
1984	0.95	1.05	0.43	0.47	0.53	0.56
1985	0.94	1.07	0.43	0.47	0.52	0.57
1986	0.83	0.77	0.39	0.37	0.48	0.46
1987	0.68	0.74	0.34	0.36	0.42	0.45
1988	0.70	0.75	0.35	0.37	0.43	0.45
1989	0.77	0.74	0.37	0.36	0.46	0.45
1990	0.74	0.78	0.36	0.38	0.45	0.46
1991	0.78	0.70	0.38	0.35	0.46	0.43
1992	0.98	0.73	0.44	0.36	0.54	0.44
1993	0.95	0.89	0.43	0.41	0.53	0.51
1994	1.11	1.11	0.48	0.48	0.58	0.58
1995	1.00	0.87	0.45	0.41	0.54	0.50
Mean			0.38	0.42	0.47	0.51
SE			0.01	0.01	0.01	0.01

shorter the assumed life span, the greater the gap between current F and threshold F—a risk prone management outcome.

Natural Mortality Rate

The instantaneous rate of natural mortality (M) was estimated using the International Council for the Exploration of the Sea (ICES) convention; that is, 5% survivorship at maximum age fol-

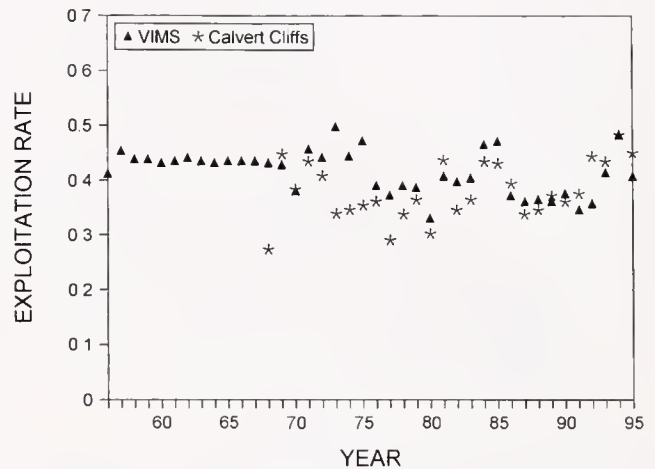


Figure 25. Estimated Chesapeake Baywide annual exploitation rate (μ) on the peeler/soft crab component of the stock using length-based estimates of instantaneous fishing mortality rates derived from the VIMS trawl (1956 to 1995) and Calvert Cliffs pot (1968 to 1995) survey data.

lowing negative exponential depletion (Anthony 1982, Vetter 1985). Using longevity of 8 y, M on Chesapeake Bay blue crabs was estimated at 0.375.

Growth

Blue crab growth (CW at age) was modeled using the formulation of von Bertalanffy (1938). The von Bertalanffy parameters were estimated using the FISHPARM (Prager 1987) fisheries software system (Fig. 29). Input data included maximum age of 8 y, and the current upper bound estimates of the size groupings for age 0 (0–59 mm), age 1 (60–119 mm) and age 2 (120–79 mm). Use of presumptive ages based on size groupings was required, because age data for blue crabs do not exist. Estimates of sizes for age 3–7 were unspecified in the model; a point estimate of 260 mm was specified for age 8. We selected 260 mm as an estimate of the mean carapace width at age 8 based on the fact that 260 mm (10 1/4 in) was the largest crab caught in Maryland that could be verified.

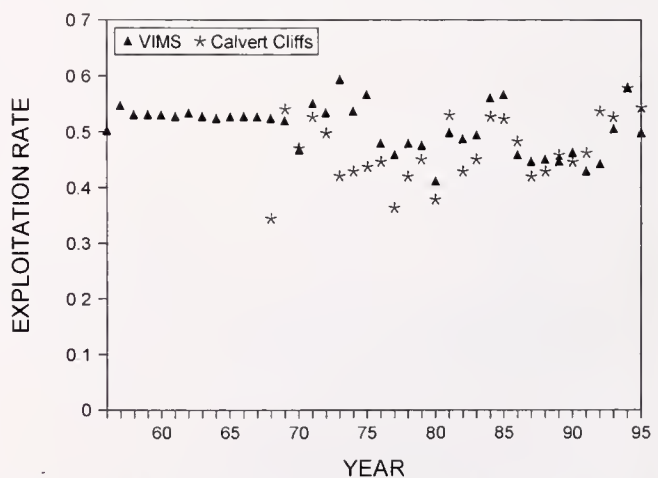


Figure 26. Estimated Chesapeake Baywide annual exploitation rate (μ) on the hard crab component of the stock using length-based estimates of instantaneous fishing mortality rates derived from the VIMS trawl (1956 to 1995) and Calvert Cliffs pot (1968 to 1995) survey data.

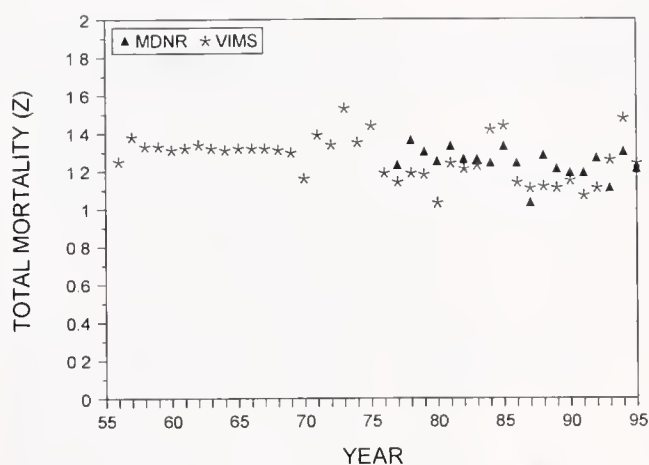


Figure 27. Estimated Chesapeake Baywide instantaneous total mortality rate (Z) based on carapace width frequency data from the VIMS (1956 to 1995) and MDNR (1968 to 1995) trawl surveys.

The capture of two large (195 mm) peeler (i.e., premolt) crabs in the 1987 CBL study, as well as reported 254 mm (10 in) peeler crabs in the Gulf of Mexico blue crab stock suggested that a maximum observed size of 260 mm was reasonable for terminal age of the *virgin* stock. Specifying this size at maximum age effectively constrained the von Bertalanffy model at its asymptotic value CW_{∞} . We disregarded many anecdotal reports of considerably larger (300+ mm CW) blue crabs in the Gulf of Mexico and the New England regions. We recognize that the continuous growth model of von Bertalanffy does not strictly reflect incremental blue crab growth that occurs via molting. We felt that this model was largely representative of mean blue crab carapace width at age and sufficient for the purpose of the analysis. The von Bertalanffy model is convenient, because estimates of K and CW_{∞} needed in population dynamic modeling are emergent properties.

Sex-specific weight at carapace width was determined using the function presented in Rothschild et al. (1992). This relationship was based on a 1992 sample of 5,000 crabs of each sex collected throughout the Chesapeake Bay by the baywide winter dredge survey. Because YPR considers female survival and growth in weight, the Rothschild et al. (1992) female weight at carapace width function was used to estimate age-specific weight (g) as:

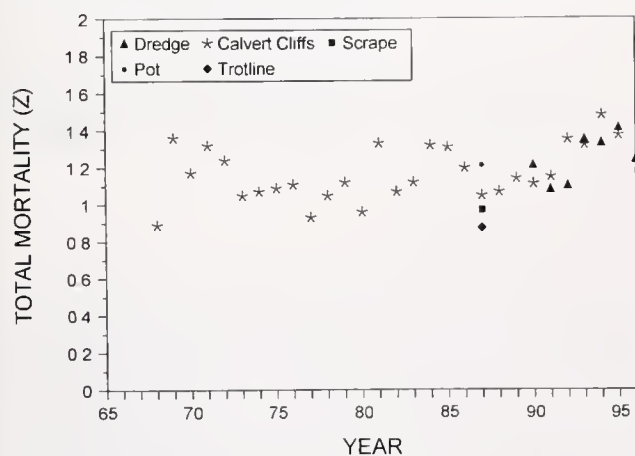


Figure 28. Estimated Chesapeake Baywide instantaneous total mortality rate (Z) based on carapace width frequency data from the Calvert Cliffs (1968 to 1995) pot, the baywide winter dredge (1990 to 1996) and the 1987 CBL pot, trotline, and scrape surveys.

$$W(g) = 0.003486 * CW^{2.1165} \text{ (mm)} \quad (7)$$

Mean Age of Stock

To reconcile the overwhelming evidence that Chesapeake Bay blue crabs live considerably longer than the current convention of 3 y, a simple life table analysis was developed using our finding of longevity of 8 y for two stock designations under varying levels of total mortality (Z). For each age (t) 1 through 8, the number of individuals surviving to the start of age $t+1$ (N_{t+1}) was derived as:

$$N_{t+1} = (N_t \cdot Pr_t \cdot e^{-(M+U)}) + [N_t \cdot (1-Pr_t) \cdot e^{-(M)}] \quad (8)$$

Our specific aim was to evaluate whether the modeled mean age and size of blue crabs in the stock was consistent with current convention and perhaps to explain the evolution of conventional wisdom that blue crabs do not live beyond 3 y of age.

The mean age of a *theoretical* stock and *observed* stock were obtained under virgin conditions ($F = 0$) and for levels of $Z = 0.8$ – 1.5 (i.e., $F = 0.425$ – 1.125). The theoretical stock is defined as all cohorts age 0–8. If survey and/or fishery data exist in which 0+ mm blue crabs are representatively sampled, Table 5 and Figure 30 provide comparative modeled results of the expected mean age and mean carapace width of the theoretical stock under virgin and contemporary fishing conditions. Survey and fishery data in which the age 0 cohort are fully represented do not exist. Consequently, we defined the observed stock as consisting of all cohorts age 1–8 (i.e., 60+ mm CW). Blue crabs of this size are sufficiently large to be represented in both research survey and fishery data, hence the term *observed*. Table 5 and Figure 30 provide the expected mean age and mean carapace width of the observed stock under virgin and contemporary fishing conditions.

Maturity at Age

Determination of female maturity at age in blue crabs was based on percentage maturity at size ogives presented in Rothschild et al. (1992), weighted by the percentage at carapace width in each age grouping. Among 65,000 female crabs examined in 1991 to 1992, Rothschild et al. (1992) found few mature individuals under 100 mm CW and essentially no immature individuals over 140 mm CW. Therefore, we assumed that all age 0 (0–59 mm CW) female blue crabs were immature and that all crabs 180 mm CW (age 3) and over were mature. Determination of the percentage mature at ages 1 (60–119 mm) and 2 (120–79 mm) was

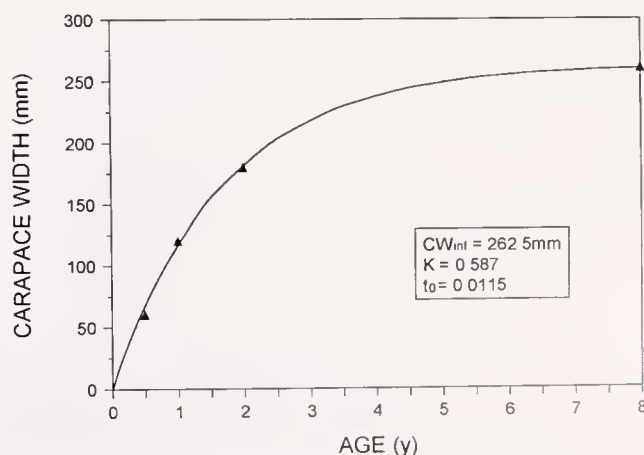


Figure 29. von Bertalanffy model of blue crab mean carapace width (mm) at age.

TABLE 5.

The proportion at age of each cohort in the theoretical stock (ages 0+) and the observed stock (ages 1+) under virgin conditions ($Z = 0.375$) and varying levels of instantaneous total mortality (Z), and the resultant mean age of the theoretical and observed stocks for each level of total mortality.

Age	Theoretical Stock								
	Total Mortality (Z)								
	0.375	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5
Proportion at Age									
0	0.324	0.438	0.456	0.470	0.483	0.494	0.503	0.512	0.519
1	0.223	0.291	0.300	0.308	0.315	0.320	0.325	0.329	0.333
2	0.153	0.148	0.143	0.138	0.133	0.127	0.122	0.117	0.113
3	0.105	0.068	0.060	0.053	0.047	0.041	0.036	0.032	0.028
4	0.072	0.031	0.024	0.019	0.015	0.012	0.010	0.008	0.006
5	0.050	0.014	0.010	0.007	0.005	0.004	0.003	0.002	0.001
6	0.034	0.006	0.004	0.003	0.002	0.001	0.001	0.000	0.000
7	0.023	0.003	0.002	0.001	0.001	0.000	0.000	0.000	0.000
8	0.016	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Mean age (y)	1.88	1.05	0.96	0.88	0.82	0.78	0.74	0.70	0.68

Age	Observed Stock								
	Total Mortality (Z)								
	0.375	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5
Proportion at Age									
1	0.329	0.518	0.552	0.582	0.609	0.633	0.655	0.674	0.691
2	0.226	0.263	0.263	0.260	0.257	0.252	0.246	0.240	0.234
3	0.155	0.122	0.111	0.100	0.090	0.081	0.072	0.065	0.058
4	0.107	0.055	0.045	0.037	0.030	0.024	0.020	0.016	0.013
5	0.073	0.025	0.018	0.014	0.010	0.007	0.005	0.004	0.003
6	0.050	0.011	0.007	0.005	0.003	0.002	0.001	0.001	0.001
7	0.035	0.005	0.003	0.002	0.001	0.001	0.000	0.000	0.000
8	0.024	0.002	0.001	0.001	0.000	0.000	0.000	0.000	0.000
Mean age (y)	2.78	1.87	1.76	1.67	1.59	1.53	1.48	1.44	1.40

based on the maturity at width (Rothschild et al. 1992) and size composition from the baywide winter dredge survey for those years (Rugolo et al. 1997). The approximate fractions of mature age 1 and age 2 female crabs were estimated at 10% and 90%, respectively.

In an effort to encompass the range of potential female maturity schedules, four maturity at age profiles were developed (Fig. 31). Although we refer to these as maturity profiles, they more accurately reflect lifetime spawning activity. Each profile contains the same initial segment for ages 0–3, which represents maturity at age. For ages 4–8 y, the proportion mature reflects spawning behavior, either constant with age after 3 y (flat-topped) or declining with age because of senescence (Dome-1 through Dome-3). The latter models reflect diminishing reproductive capacity after the first year of spawning as a result of the theorized one-time mating event upon maturation.

Rugolo et al. (1997) discussed the biological implications of this range of maturity profiles and their influence on the estimation of biological reference points. In short, the assumption of lifetime spawning potential (flat-topped) is most risk averse for reference point calculation; however, it is the most biologically unrealistic, because it is generally accepted that female blue crab reproductive

capacity diminishes after first spawning. In contrast, the maturity schedule designated as Dome-3 is most consistent with current understanding of blue crab reproductive biology and also most risk prone in terms of estimating threshold fishing mortality rates. We selected Dome-2 for subsequent YPR modeling. This choice reflected a compromise between beliefs that female blue crabs demonstrate sharply declining reproductive capacity with age and our desire to be risk averse in the estimation of biological reference points for the Chesapeake Bay stock.

Biologic Reference Points

Yield per Recruit Analysis

To assess stock conditions at varying levels of fishing mortality (F) and to estimate biological reference points, we developed a YPR model following the method of Thompson and Bell (1934). For each level of F , model output included total stock and spawning stock size per recruit (weight and number), and percentage of maximum spawning potential (%MSP). A range of fishing mortality rates from $F = 0$ –2.0 were examined in each model run. In all model formulations, M was constant with age at 0.375; maximum age was 8 y. Lacking empirical evidence to the contrary, the

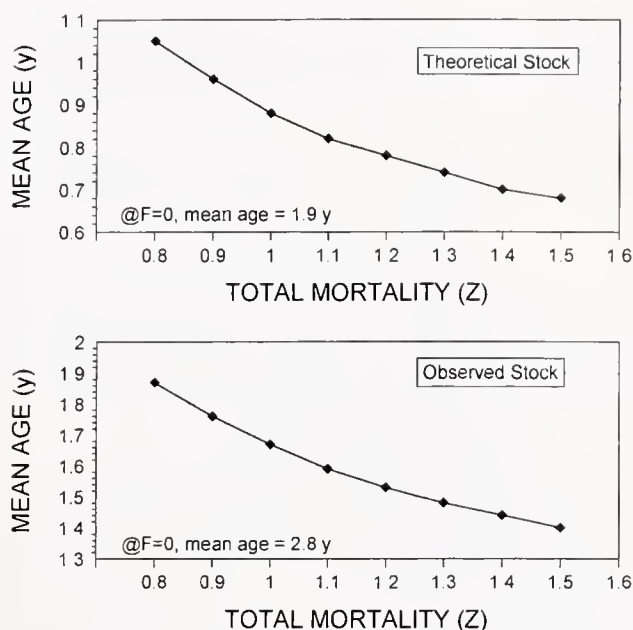


Figure 30. Mean age of the Chesapeake Bay population of blue crabs under virgin conditions ($F = 0$) and varying levels of instantaneous total mortality (Z) for the *theoretical* stock (ages 0+) and *observed* stock (ages 1+).

mean weight of the stock was set equal to that in the harvest and derived from the von Bertalanffy mean width at age (Rugolo et al. 1997) and width-weight relationships (Rothschild et al. 1992). The age-specific pattern of recruitment to fishing mortality was estimated based on size-specific selectivity of the principal harvest gears, minimum size regulations, and the relative contribution of each gear to the baywide landings (Rugolo et al. 1997). The resulting PR schedule was 0.10, 0.75, 0.95, and 1.0 for ages 0, 1, 2, 3–8, respectively. The proportions of M and F occurring before spawning were set to 0.75 and 0.635, respectively (Rugolo et al. 1997).

Four YPR model configurations were developed, one for each maturity at age profile providing YPR, spawning stock biomass per recruit (SSB/R), %MSP values, and a set of biological reference points. Rugolo et al. (1997) present the results of each model

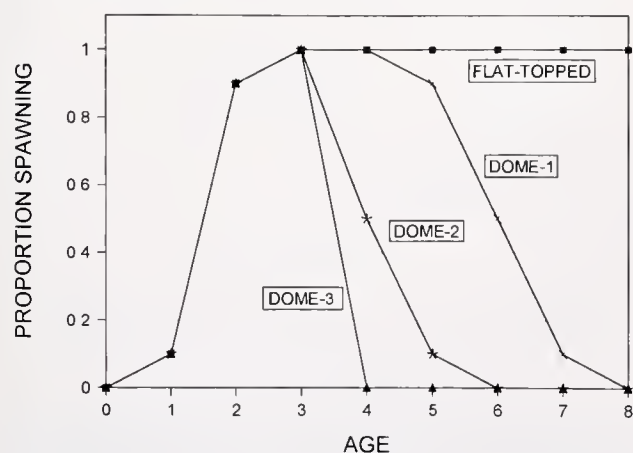


Figure 31. Maturity at age (spawning activity) profiles for female Chesapeake Bay blue crabs.

run and discuss the consequences on reference point calculation of the four maturity profiles and longevity 4, 6, and 8 y. Here, we present only the results of the most biologically reasonable maturity schedule, the Dome-2 profile. Estimates of threshold fishing mortality rates $F_{0.1}$, F_{MAX} , and $F_{\%MSP}$ (5–20%) are presented in Figure 32.

Spawning Stock Abundance per Recruit Analysis

Spawning stock abundance per recruit (SSA/R) output from the YPR analysis was combined with S–R data to estimate F_{HIGH} , F_{MED} , and F_{LOW} biological reference points. When S–R data are represented in numbers of spawning stock and recruits, the slope of a line drawn from the origin through the scatter plot provides an index of the number of recruits produced from a given number of spawning individuals. The slope can be used in combination with YPR modeling to estimate threshold fishing mortality rates F_{HIGH} , F_{MED} , and F_{LOW} .

We selected the historic VIMS trawl survey as a source of recruit and spawner metrics, because it was the longest time series record of these measures; thereby, providing the widest potential range of recruit and spawner values, and because they were found to provide the best fit to the Ricker S–R model (Rugolo et al. 1997). The VIMS survey index of recruits (<60 mm CW) was plotted against the index of female spawning stock (≥ 130 mm CW) for this analysis (Fig. 33).

The median line bisects the field of points and is considered to represent a level at which the stock is likely to sustain itself (ICES 1983). Using this median recruit-to-spawner ratio we estimated F_{REP} , the level of fishing mortality that allows the stock to persist by providing sufficient recruitment, on average, to replace losses caused by all sources of mortality. F_{REP} as a biological reference point implicitly considers the vagaries of density-independent effects on stock stability. Two additional lines were drawn through the field of S–R points so that either 25 or 75% of the points were above the line. The slopes of these lines reveal F_{HIGH} and F_{LOW} threshold mortality rates, respectively (Fig. 33). The value F_{HIGH} is generally thought to be too high for a stock to sustain itself in the long term, especially for r-selected species, such as blue crabs, because only 25% of the production of new individuals exceeds this value on average. The value F_{LOW} is a risk averse reference

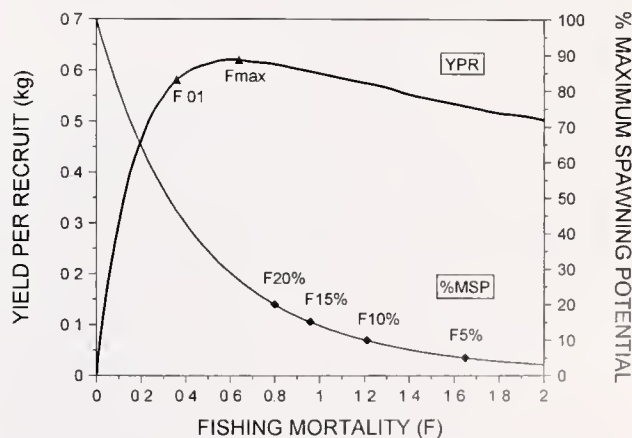


Figure 32. Yield per recruit and percentage maximum spawning potential for Chesapeake Bay blue crabs based on the method of Thompson and Bell (1934) under the Dome-2 maturity profile. Biological reference points $F_{0.1}$, F_{MAX} , and $F_{\%MSP}$ (5–20%) are indicated.

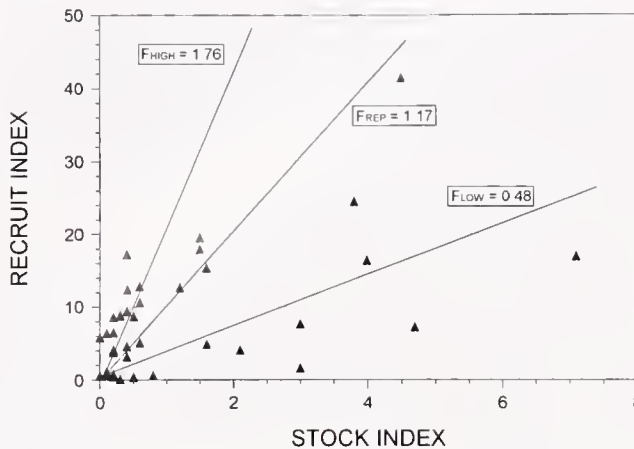


Figure 33. Spawning stock abundance per recruit analysis under the Dome-2 maturity profile based on the VIMS trawl survey female spawning stock index (≥ 130 mm CW) and the age 0 (0–59 mm CW) recruitment index for years 1956 to 1995. Biological reference points F_{HIGH} , F_{REP} , and F_{LOW} are indicated.

value that would theoretically avoid growth and recruitment overfishing. For the blue crab, F_{LOW} was found to be less than $F_{0.1}$ and F_{MAX} , thus a portion of the potential equilibrium yield would be theoretically lost to fisheries operating at this F . Rugolo et al. (1997) compare SSA/R modeling results for all four maturity at age profiles in terms of F_{HIGH} , F_{MED} , and F_{LOW} threshold values.

Gear Saturation Analysis

In this analysis, we examined the essential operational elements of the Chesapeake Bay blue crab fisheries from 1945 to 1995. Despite a near fivefold increase in total directed fishing effort since 1945 (Fig. 13), annual exploitation rate (Fig. 25, 26) has varied without trend. This dramatic rise in effort, coupled with relatively stable harvest (Fig. 14), resulted in a decline in baywide CPUE since 1945 (Fig. 15). Although recent estimates of F demonstrate a slightly increasing trend (Table 4), this increase is within the range of variability in these values since 1956. Assuming constant q and stock size, fishing mortality would have been expected to rise since the mid-1950s in proportion to the observed rise in effort. Because estimates of F did not increase in proportion to increases in effort, the indication was that catchability has not remained constant since the mid-1950s.

We considered that the assumption of constant q has been violated for the blue crab fishery owing largely to the use of static capture gear as the primary means of harvest (Rugolo et al., this volume). The mechanisms responsible for the lack of proportionality between F and f have not previously been examined for the Chesapeake Bay blue crab fisheries. Varying catchability for several finfish species has been attributed to changes in stock availability, gear selectivity, gear saturation, and changes in stock abundance (Pope and Garrod 1975). If q for blue crabs declined as commercial fishing effort increased, we hypothesized that gear saturation was the principal cause, because the alternatives were either unreasonable or counterindicated by this assessment. The use of static gear in crustacean fisheries requires the target organism to enter the gear voluntarily, which is in contrast to the capture strategy of such mobile gear fisheries as trawls or purse seines.

We examined the hypothesis of gear saturation in the Chesapeake Bay blue crab fishery and its effects on catchability and

fishing mortality. Nominal fishing effort and estimates of historic fishing mortality rates were subject to the analysis. Annual catchability coefficients (q) were estimated as the ratio of fishing mortality to effort:

$$q = F/f \quad (9)$$

To test the hypothesis that q is inversely related to nominal fishing effort (f), the value of q was related to f by a log-log regression as:

$$\log(F/f) = \log a + B \log(f) \quad (10)$$

In the absence of independent estimates of catchability, Eq. (10) has clear statistical problems, because fishing effort is contained in both sides of the equality. For this reason, we further examined the hypothesis of nonconstant q by a simple linear regression between fishing mortality (F) and effort (f) by:

$$F = a + bf \quad (11)$$

If the catchability coefficient (q) is constant, the slope (b) of Eq. (11) would be significantly greater than zero based on a paired t -test at $n-2$ degrees of freedom. This outcome would indicate that fishing mortality changes proportionally with effort at the rate q . Conversely, if gear saturation is operative, the slope (b) should either not differ significantly ($p < .05$) from zero or should differ from zero in a negative direction. Either of these two latter outcomes would indicate that fishing mortality is not proportional to effort as specified in Eq. (9) over the range of effort values examined.

Rugolo et al. (1997) also test the hypothesis of nonproportionality between F and f resulting from gear saturation in the New England American lobster (*Homerus americanus* Milne-Edwards) fisheries. This fishery has undergone similar historical rises in directed fishing effort and a nonproportional rise in fishing mortality. The results of the log-log regression analysis of catchability (F/f) and effort (f) in which q is derived from the ratio of length-based F s to nominal effort, are presented in Figures 34 and 35. Results of the linear regression analysis of F on f using length-based F s and nominal effort are presented in Figures 36 to 38.

RESULTS AND DISCUSSION

Adult Stock

To gain insight into and to gauge the status of the adult stock, fisheries-independent time series data on relative blue crab abun-

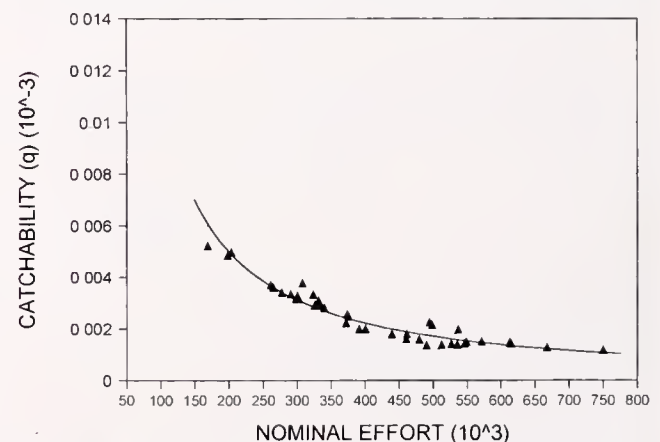


Figure 34. Observed log-log regression of fishery catchability (q) and total baywide nominal fishing effort for 1956 to 1995. Catchability was estimated from the ratio of VIMS trawl survey length-based estimates of instantaneous fishing mortality (F) and nominal effort (f).

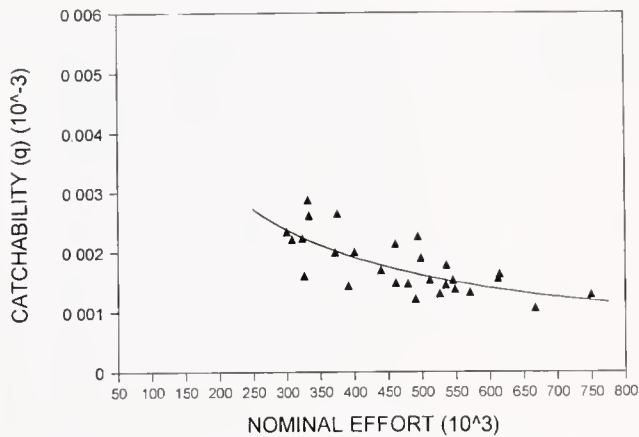


Figure 35. Observed log-log regression of fishery catchability (q) and total baywide nominal fishing effort for 1968 to 1995. Catchability was estimated from the ratio of Calvert Cliffs pot survey length-based estimates of instantaneous fishing mortality (F) and nominal effort (f).

dance were compiled. Inspection of these data provided an initial, first-order approximation of the current status of the adult stock and evidence of the change in that status over the last four decades. These abundance data demonstrated inherent internal variability over the long term and suggested that the baywide adult blue crab stock is at long-term average levels of abundance measured since 1956.

The total age 1+ index of relative abundance from the baywide winter dredge survey (1990 to 1996, Fig. 1) averaged approximately 5.5 crabs/1,000 m^2 for sexes combined with a standard error of the mean (se) = 1.1. Age 1+ CPUE in this survey ranged from a low of 3.7 in 1995 to a high of 8.8 in 1991. These data revealed a slightly declining trend in age 1+ abundance since 1990; however, this trend was not dramatic when viewed in the long term. The 1996 age 1+ index of 4.9 (sexes combined) was not significantly less than the 7 y average of 5.5 ($p < .05$). Pronounced differences between age 1+ male and female relative abundance were not shown by these data. The trend in age 2+ relative stock abundance for sexes combined revealed similar results (Fig. 2). From 1990 to 1996, the average age 2+ abundance for male and

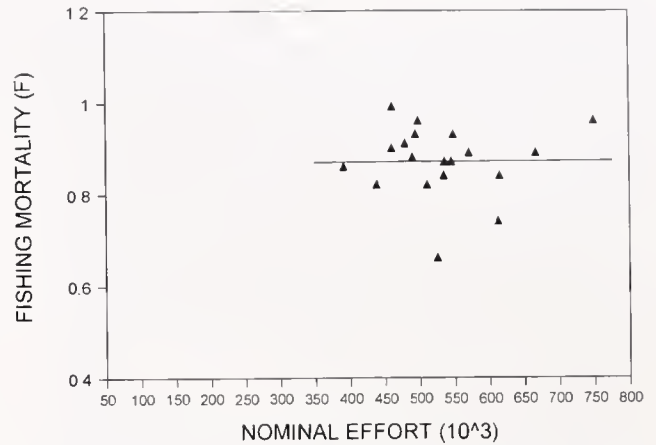


Figure 37. Observed linear regression of instantaneous fishing mortality (F) and total baywide nominal fishing effort (f) for 1977 to 1995. Fishing mortality rate (F) was derived from the MDNR trawl survey length-based estimates of instantaneous total mortality (Z).

female combined was approximately 3.0 crabs/1,000 m^2 , ranging from a low of 1.4 in 1995 to a high of 5.3 in 1991. The 1996 index of 3.0 was at the long-term average.

The MDNR blue crab trawl survey provided regional indices of relative abundance for crabs age 0 and older. Derivation of an internally consistent measure of CPUE from this survey was problematic, because some systems and/or months were not sampled in many years. Relative abundance of male and female age 1+ blue crabs revealed widely fluctuating levels during 1977 to 1995 (Fig. 3). Age 1+ CPUE (mean number/tow) from this survey steadily declined from 1984 with the 1995 value for male (3.7) and female (3.7) crabs below the respective long-term averages since 1977 (7.0, se = 0.9 for males, and 8.4, se = 1.0 for females). Results of this survey suggested that the decade of 1980 was a period of relatively high blue crab abundance, which was preceded and followed by lower age 1+ abundance. The average male (8.1, se = 1.0) and female (5.7, se = 0.7) relative abundance from 1990 to 1995 did not differ significantly from the respective means during 1977 to 1995.

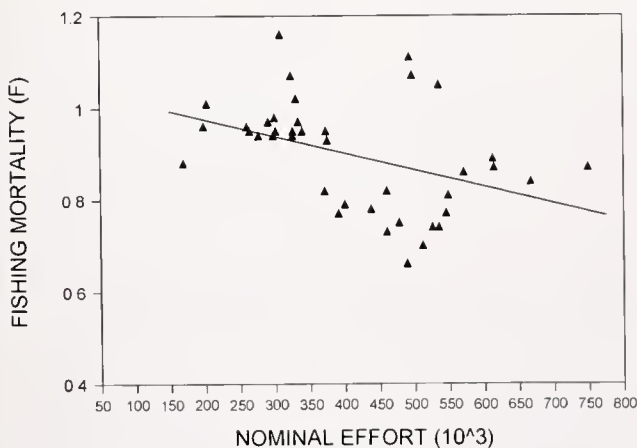


Figure 36. Observed linear regression of instantaneous fishing mortality (F) and total baywide nominal fishing effort (f) for 1956 to 1995. Fishing mortality rate (F) was derived from the VIMS trawl survey length-based estimates of instantaneous total mortality (Z).

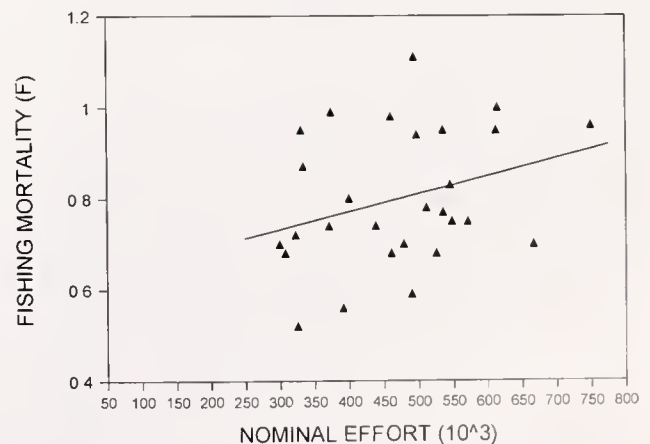


Figure 38. Observed linear regression of instantaneous fishing mortality (F) and total baywide nominal fishing effort (f) for 1968 to 1995. Fishing mortality rate (F) was derived from the Calvert Cliffs pot survey length-based estimates of instantaneous total mortality (Z).

During 1968 to 1995, the VIMS trawl survey corrected index of relative age 1+ abundance (sexes combined) averaged 5.4 (se = 0.8) and ranged from a low of 0.4 in 1975 to a high of 17.6 in 1990 (Fig. 4). Both the 1990 age 1+ index (5.5) and the 1990 to 1995 average abundance (6.8, se = 2.3) lie within the 95% confidence limits (5.4–8.9) on the 1977 to 1995 mean. Inspection of trends from this survey also suggest that the decade of 1980 was a period of sustained above average levels of age 1+ blue crab abundance.

The trends in age 1+ male and female blue crab abundance measured by the Calvert Cliffs peeler pot survey revealed similar patterns of abundance to those of other fishery-independent surveys (Fig. 5). Relative male and female age 1+ CPUE (mean number/pot) averaged 2.9 (se = 0.3) and 3.1 (se = 0.4) respectively from 1968 to 1995, ranging from a low of 0.6 (1968) for males and 0.3 (1968) for females, to a high of 8.6 in 1981 for males and 10.4 in 1981 for females. The 1995 male (1.9) and female (1.6) age 1+ abundance were below the respective lower 95% confidence limit (2.3 for males and 2.4 for females) on the long-term means from 1968 to 1995. This survey similarly showed that the 1980s was a period of above average abundance for both male and female age 1+ crabs. Abbe and Stagg (1996) and Rugolo et al. (1997) found that the trend in relative blue crab abundance from the Calvert Cliffs survey and the Maryland blue crab commercial harvest were highly correlated. During 1968 to 1995, the Calvert Cliffs age 1+ abundance index (sexes combined) was correlated with the Maryland pot and Maryland total harvest ($r^2 = 0.72$ and 0.70 , respectively, $p < .0001$), and with the Maryland pot CPUE ($r^2 = 0.88$, $p < .0001$) (Rugolo et al., this volume). The change in relative abundance in the exploitable stock measured by this survey was found to account for the majority of the change in Maryland commercial yield between 1968 to 1980 and 1981 to 1995 (Rugolo et al. 1997).

The historic Smith Island scrape study (1948 to 1972; Fig. 6) and the Calvert Cliffs peeler pot survey (1968 to 1995; Fig. 5) provided consistent sampling of age 0+ and age 1+ blue crabs respectively. For the 5 years in which both surveys were conducted (1968 to 1972), the respective indices of blue crab relative abundance were found to be highly correlated, and the trend in age 1 CPUE from the Smith Island study was generally representative of the pattern of commercial blue crab harvest in these years (Rugolo et al. 1997). Based on reported harvest, relatively low stock abundance was suggested during 1951 to 1960. Exploitable stock abundance increased steadily from 1964 to 1995 with the notable exception of 1968 (Fig. 6). When all sources of data are considered, such as reported commercial harvest and the Calvert Cliffs and Smith Island surveys, 1968 represented the lowest recorded blue crab stock abundance in Maryland since 1945. Based on the strong concordance in the Calvert Cliffs age 1+ and Smith Island age 1 indices during 1968 to 1972 ($r^2 = 0.96$), we developed an approximate 50 y time series of relative abundance by merging age-specific indices according to the derived functional relationship $CC_{1+} = 8.97^{-4} \times SI_1^{1.41}$ (Rugolo et al. 1997). In this approach, we chose to express the historical Smith Island age 1 index in the currency of Calvert Cliffs age 1+ abundance, because the latter study is on-going, and these age groupings were most representatively sampled by the respective surveys. The combined Smith Island and Calvert Cliffs survey data revealed higher relative adult stock abundance during the period 1981 to 1995 than during 1948 to 1980 (Fig. 7). The extended data series suggested that the periods between 1951 to 1960 and 1968 to 1980 were

years in which blue crab abundance was below the long-term average from 1948 to 1995.

Estimated baywide hard crab population abundance (Fig. 8) from 1956 to 1995 varied widely, with the decade of 1980 seen as a relatively above average period. From 1956 to 1995, the mean population abundance was approximately 385×10^6 hard crabs (se = 17.3×10^6). The 1995 estimate of 399×10^6 crabs was above the 1956 to 1995 mean, although within its 95% confidence limits. Considering the inherent interannual variability expected from a species such as blue crabs, and variability in the sampling programs, there is concordance in the trends of relative stock abundance measured by the five fishery-independent surveys. These time series data indicate that the Chesapeake Bay adult blue crab stock is at long-term average levels of abundance measured during 1956 to 1995. Trends in relative stock abundance do not reveal a contemporary stock demise, as anecdotal information suggested, or a systematic long-term decline in abundance. The decade of the 1980s was consistently a period of above average stock abundance, which was followed by a return to long-term average levels. Using the derived annual exploitation rate estimates, the number of blue crabs in the adult baywide population is at or slightly above the long-term mean estimated since 1956.

Juvenile Recruitment

Among the fishery-independent surveys examined in this phase of the analysis, the baywide winter dredge survey and the Smith Island scrape study for age 0, and the Calvert Cliffs peeler pot survey for age 1 were considered to provide representative measures of relative juvenile abundance. The baywide winter dredge survey provides the broadest geographic coverage. Limited geographic coverage is not necessarily fatal for the purpose of inter-annual comparisons of trends if the survey sampling protocol is internally consistent among years and if the survey takes a representative sample of the available stock each year. The MDNR trawl survey age 0 relative abundance index is potentially less reliable than that from the other surveys because of the temporal/spatial sampling problems noted, and age 0 crabs are not fully recruited to the gear.

Results from the baywide winter dredge survey revealed that the 1996 age 0 abundance index of 12.0 was the highest on record for sexes combined and nearly twice that of the next highest year (1990) at 6.0 crabs/1,000 m² (Fig. 9). During 1990 to 1996, juvenile blue crab recruitment from this survey has been relatively constant. Juvenile recruitment measured by the MDNR trawl survey (Fig. 10) demonstrated widely varying levels of year class strength between 1977 to 1995. The relative abundance of male and female age 0 crabs showed a generally increasing trend from 1987 to 1993 although the 1995 index was one of the lowest on record. Average (1977 to 1995) male and female mean number/tow were 2.8 (se = 0.5) and 3.4 (se = 0.6), respectively. Two periods of average to above-average juvenile abundance were seen, the first in the mid-1980s and the second during the late 1980s to early 1990s. During 1968 to 1995, the VIMS corrected age 0 blue crab abundance index for sexes combined varied without trend and ranged from a low of 0.5 in 1974 to a high of 18.1 in 1990 (Fig. 11). Long-term (1968 to 1995) average 0 group relative abundance was 5.6 (se = 0.8). Although the 1995 index of 4.5 was approximately 20% below the mean, it was approximately 15% above average 1968 to 1979 recruitment of 3.9 (se = 1.0) and within the 95% confidence limits (4.0–7.2) on mean 1968 to 1995 recruitment.

Juvenile blue crab recruitment (age 1) measured by the Calvert Cliffs peeler pot survey in 1968 to 1995 was fairly consistent throughout the time series, with a period of high abundance in the mid-1980s (Fig. 12). Relative male and female age 1 mean number/pot averaged 0.7 (se = 0.1) and 0.4 (se = 0.1), respectively, from 1968 to 1995 and ranged from a low of 0.07 (1968) for males and 0.02 (1968) for females to a high of 2.9 in 1981 for males and 1.6 in 1981 for females. These data suggested a slightly increasing trend in male and female recruitment to the stock, with the 1995 male (0.9) and female (0.4) relative abundances at 20.5% above and 18.6% below the respective long-term means. Relative abundance of age 0 blue crabs for sexes combined measured by the Smith Island scrape study varied without trend during the 25-y period (Fig. 6). Juvenile recruitment peaked in 1950 and declined to relatively constant levels thereafter, with the exception of 1968. As shown in the Calvert Cliffs survey, 1968 represented the lowest juvenile abundance recorded in Maryland since 1948. Estimates of absolute peeler/soft crab abundance varied without trend during 1956 to 1995 and suggested that 1995 recruitment (39.2×10^6 crabs) was above the long-term mean of 35.8×10^6 crabs (se = 2.7×10^6) (Fig. 8). Caution is urged in interpreting these results in that the baywide peeler/soft crab harvest, upon which these estimates are derived, is not as well reported as hard crab harvest. If internal biases in these data are random and not systematic or directional, these stock size estimates can provide a basis for interannual comparisons.

Overall, the historic juvenile recruitment signal was noisy in the long-term, as expected, considering the unique population dynamics of the species and the role of density-independent effects on recruitment success. Current production of new individuals and their recruitment to the stock can be considered to be at long-term average levels for the last four decades. Trends in these data do not provide compelling evidence of a failure in juvenile recruitment, which was suggested prior to initiating this research.

Fishery Yield and Catch per Unit Effort

Historic commercial time series data from the principal baywide fisheries were examined in this phase of the study. Based on recent time series of harvest and effort data, Maryland pot and trotline CPUE has remained fairly stable over the 14-y time period (1982 to 1995), and no evidence of a decline in performance of these fisheries was observed (Fig. 16). Mean CPUE (kg/pot/month) for the pot fishery increased from 12.9–19.6 from 1982 to 1985 and declined to 10.2 in 1994. Trotline CPUE (mean kg/trotline-h/mo) increased between 1982 to 1985 from 10.2–17.1 and remained fairly constant thereafter. The total yield of Chesapeake Baywide commercial fisheries has varied without trend since 1945 (Fig. 14). Baywide harvest since 1945 seemed to cycle around the long-term mean, with peaks in 1950, 1966, 1981, and 1993 and troughs in the mid to late 1950s and late 1970s to early 1980s. Baywide data from 1945 to 1995 suggest an approximate fivefold increase in total nominal directed effort (Fig. 13). The fact that baywide harvest has remained relatively stable since 1945, coupled with a dramatic rise in fishing effort, was reflected in decreasing CPUE in the baywide fishery (Fig. 15). Baywide commercial CPUE declined rapidly from 1947 to 1967 and has been slightly noisy and without trend since the late 1960s. These data indicate that the Chesapeake Bay blue crab fishery experienced hyperdepletion in which relatively constant harvest is obtained despite increasing levels of directed effort (Rugolo et al. 1997). In their

study, Rugolo et al. (1997) examined hyperdepletion in the fisheries for *C. sapidus* and *H. americanus*. We provide here evidence that coincident with the rise in fishing effort for the baywide fishery, there was a decline in q because of gear saturation.

From 1981 to 1995, the Maryland commercial harvest data (Table 2) suggested a period of increased yield. The baywide commercial landings (Fig. 14) also revealed increased yield commencing in the decade of 1980; although their magnitude was not excessive when viewed in the long-term context, especially compared to the yields of the mid-1960s. cursory examination of these harvest data suggested that the increase in yield in the 1980s was an artifact of a coincident reporting system change in Maryland in 1981. Rugolo et al. (this volume) provided evidence that the increase in yield to the Maryland fisheries after 1980 resulted from an increase in underlying stock abundance. They examined two measures of blue crab abundance that were independent of the Maryland harvest data or the reporting system change; namely, the Calvert Cliffs age 1+ stock abundance index and the Potomac River harvest and effort time series data. Commercial blue crab harvest from the Potomac River (Fig. 17) demonstrated increases in yield after 1980 analogous to those seen in Maryland landings without a change in the Potomac River reporting system. The mean percentage change in the Potomac River blue crab harvest pre-1981 versus 1981 and later accounted for all the percentage change in the Maryland reported harvest between these periods (Rugolo et al., this volume). Second, the change in relative abundance in the exploitable stock (127+ mm) measured by the Calvert Cliffs pot survey accounted for 96% of the percentage change in Maryland commercial yield before and after the change in reporting system. The trends in abundance between the Calvert Cliffs survey data and the Maryland commercial harvest have been shown to be highly correlated (Abbe and Stagg 1996, Rugolo et al. 1997, Rugolo et al., this volume), and it seems that these data are internally consistent. The relative abundance of age 1+ blue crabs measured by the MDNR trawl survey (Fig. 3) and the VIMS trawl survey (Fig. 4) also suggested increased abundance in the exploitable stock in the 1980s. These measures of blue crab stock abundance, which were independent of the Maryland harvest data, provided clear and compelling evidence that the increase in yield to the Maryland fisheries after 1980 resulted from an increase in the underlying abundance of the stock targeted by the fisheries at a near constant rate of exploitation.

Surplus Production

Schaefer (1954) and Fox (1970) surplus production models were fit to commercial blue crab harvest and total directed nominal effort data (Table 2) for the purpose of estimating maximum sustainable yield (MSY) for the baywide commercial fisheries. Nominal baywide directed effort showed a general upward trend, reflecting increased interest in the blue crab fishery during the last 50 y (Fig. 13). Changes in reporting methods and difficulties posed by the absence of gear-specific efficiency measures increase the uncertainty in these effort data; however, they are consistently derived and are believed to reflect the general trends in the fishery over time. Estimates of equilibrium yield derived from the Schaefer and Fox surplus production models were similar. Estimated MSY from the Schaefer model was 38.5×10^6 kg ($B_{\infty}q = 0.67$, $B_{\infty}q/k = 5.7 \times 10^7$, $r^2 = 0.02$) while that from the Fox model was 37.2×10^6 kg ($B_{\infty}q = 0.88$, $q = 8.2 \times 10^{-3}$, $K = 4.6 \times 10^3$, $r^2 = 0.17$) (Fig. 18,

19). These MSY results were somewhat higher than Tang's (1983) estimate, reflecting increases in harvest and effort in the baywide commercial fisheries since his work was completed.

Both surplus production model fits were poor, in part because historical fishing effort has not sufficiently eroded stock biomass, an outcome requisite to good fit to these model forms. These results provided some insight, nonetheless, into the dynamics of the commercial fisheries and suggested that baywide commercial MSY is $37\text{--}38.5 \times 10^6$ kg. These values of commercial yield were approximated or exceeded in the mid-1960s and largely exceeded after 1980. The 1995 baywide commercial yield of 33.5×10^6 kg was slightly below equilibrium yield.

Stock-Recruitment

We fit the Ricker (1954) stock-recruitment model to six adult stock and seven recruit metrics for Chesapeake Bay blue crab. Results of this analysis demonstrated weak or no relationships between adult stock and subsequent recruitment. The various S-R models revealed low reliability of fit and questionable utility for predicting recruitment success at specified levels of spawning stock biomass. The two best model fits resulted from the combination of the VIMS trawl survey female spawning stock index (≥ 130 mm CW) in year t versus the VIMS survey age 0 index in year $t + 1$ ($r^2 = 0.27$) (Fig. 20) and the MDNR trawl survey age 0 index in year $t + 1$ ($r^2 = 0.21$) (Fig. 21). Examination of the VIMS and MDNR trawl survey indices of abundance revealed higher correlations among age classes within year than for a year class between years (Rugolo et al. 1997). Thus, resulting indices of abundance from these surveys in any year might be influenced by availability to the gear as well as by the underlying abundance of the stock. Density-independent effects with potential influence on blue crab recruitment success were examined for their contribution to model variability. A method described in Tang (1985) was used to incorporate environmental measures that correlate with recruitment into the Ricker S-R model. We examined measures of bottom water temperature, salinity and dissolved oxygen, and cumulative river flow from the Susquehanna River through the mouth of the Chesapeake Bay. Tang (1985) was able to improve the fit of his model of winter dredge fishery landings in year t (stock) against Virginia commercial landings in year $t + 1$ (recruits) from $r^2 = 0.34$ to 0.69 by incorporating radiant energy, stream flow, and minimum and average water temperatures. We found that the environmental factors that correlated with the two recruit metrics were not significantly correlated with the density-independent parameter (a) of the Ricker model through multiple regression analysis (VIMS age 0s, $p = 0.7$; MDNR age 0s, $p = 0.8$). We are further analyzing density-independent effects that we consider to be more influential on blue crab early life history survival or recruitment to the stock. These effects include wind vectors that govern blue crab larval transport, radiant energy that may increase productivity, and therefore minimize the match-mismatch between larval/juvenile crabs and their prey, and surface temperature that should influence early life stages more than bottom conditions.

Exploitation Rates

We developed a measure of relative exploitation rate (μ_{REL}) and estimated absolute exploitation rate (μ_{ABS}) on the peeler/soft crab and hard crab components of the stock. Relative exploitation rate provided a means for assessing the time series trend in exploitation, while absolute exploitation rate is the fraction of the

stock removed by the fishery each year. Relative exploitation rate μ_{REL} , calculated using the Calvert Cliffs age 1+ and age 2+ indices as measures of stock biomass, varied without trend since 1968 (Fig. 22). These results suggested that exploitation decreased slightly from the late 1960s through the mid-1980s. Subsequently, μ_{REL} increased, but not to unprecedented levels; higher μ_{REL} occurred in 1970 and 1987. Relative exploitation rate based on the VIMS trawl survey age 1+ and age 2+ abundance indices did not seem to have changed significantly during 1968 to 1995 (Fig. 23). The 1968 and 1974 (age 1+) and the 1975 (age 2+) μ_{REL} values were comparatively high, which artificially dampened variability in μ_{REL} in the remaining years. These data suggested that a slight rise in μ_{REL} occurred in 1993 to 1994; in 1995 exploitation returned to previous levels. Using the MDNR commercial harvest and the Calvert Cliffs age 1+ and age 2+ CPUE data, μ_{REL} in the Maryland fisheries seemed to have risen since the mid-1980s, as compared to the 1970s (Fig. 24). Relative exploitation was slightly higher in recent years followed by a decline in 1995. We recognize that μ_{REL} based on an index of exploitable stock biomass might be less robust than the direct enumeration of fishing mortality rates. Although simplistic, this analysis provided no definitive evidence of an unprecedented rise in exploitation rate on the Chesapeake Bay blue crab stock in recent years.

Absolute exploitation rates were estimated on the peeler/soft crab and hard crab components of the stock for years 1956 to 1995 using the length-based estimates of total mortality (Z) and the partial recruitment of each stock component to the fully recruited rate of fishing mortality (F) (Table 4). For the peeler/soft crab stock (primarily age 1 crabs), μ_{ABS} ranged from a low of 0.33 in 1980 to a high of 0.50 in 1973 based on the VIMS survey (1956 to 1995) (Fig. 25). Mean μ_{ABS} for these data was 0.42 (se = 0.006). Using the Calvert Cliffs survey data (1968 to 1995), μ_{ABS} on this stock component ranged from 0.27 (1968) to 0.48 (1994), with a mean $\mu_{ABS} = 0.38$ (se = 0.01) (Fig. 25). Annual exploitation rates on the baywide peeler/soft crab stock were noisy over the long-term, with the majority of μ_{ABS} values falling between 0.35–0.45. No marked increase in μ_{ABS} was evident in these data, which would indicate over exploitation of the stock. Current μ_{ABS} was below the threshold exploitation rate of 0.46 for the peeler/soft crab.

For the hard crab stock (primarily age 2+), μ_{ABS} ranged from a low of 0.41 in 1980 to a high of 0.59 in 1973 based on the VIMS survey (Fig. 26). Mean μ_{ABS} for these years was 0.51 (se = 0.007). Using the Calvert Cliffs survey data, exploitation rates on the hard crab stock ranged between 0.34 (1968) and 0.58 (1994), with a mean $\mu_{ABS} = 0.47$ (se = 0.01) (Fig. 26). The annual rate of exploitation experienced by the hard crab stock was similarly noisy in the long term, with the majority of values falling between 0.40–0.50. As seen for the peeler/soft crab stock, μ_{ABS} increased slightly between 1991 to 1994 and declined in 1995 toward average levels. No recent increase in μ_{ABS} that would indicate over-exploitation of the hard crab stock was observed. Both current and historical μ_{ABS} fall below the threshold μ_{ABS} of 0.61 for the hard crab stock.

Total and Fishing Mortality Rates

Instantaneous rates of total (Z) and fishing mortality (F) were estimated for years 1956 to 1995 using length-based analysis (Table 4). The time series Z estimates from the Calvert Cliffs, VIMS, and MDNR fishery-independent surveys demonstrated no

significant increasing trends since 1956, which would imply a recent increase in fishing mortality on the stock (Fig. 27, 28). Within the range of variability in the Calvert Cliffs and baywide winter dredge survey estimates of Z , marginal increases in total mortality were observed for 1990 to 1994, followed by a decline in 1995. Using Calvert Cliffs research survey data, the time series of total mortality was observed to be slightly noisy and cyclic from 1968 to 1995. All estimates of Z were less than 1.50; no increasing trend in total mortality was observed. Based on the baywide winter dredge survey data, a slight increasing trend in Z was detected from 1992 to 1995, followed by a decline to more average levels in 1996. All winter dredge survey-based estimates of Z were less than 1.40. For the VIMS trawl series data, a slight rise in Z was observed after 1991, followed by a decline in 1995 to average levels. The recent highest Z from these data was in 1994 at 1.49, with one year (1973) higher than this value since 1956. All VIMS survey-based estimates of Z were less than 1.55. Using length data from the MDNR trawl survey (1977 to 1995), the resulting time series of Z was essentially flat and trendless. All estimates of Z from this survey were less than 1.40. Length frequency data from the 1987 CBL study showed that Z was less than 1.20.

Fishing mortality rates derived from the length-based analysis demonstrated no significant directional trends over the period of record (1956 to 1995) (Table 4). Any short-term rise in F was not to unprecedented levels nor would be considered excessive for the stock. On average, the time series of annual fishing mortality rates was slightly noisy, varying within a narrow range in the long term, principally between 0.8–1.0. Absolute values of F during 1956 to 1995 ranged from 0.50–1.16, corresponding to annual exploitation rates of approximately 33 and 59%, respectively. All recent and current F s were less than the threshold fishing mortality rate ($F_{10\%}$) deemed appropriate for this species. No evidence of overexploitation, specifically recruitment overfishing, has occurred. Current F is above that which would maximize YPR.

Life History Characteristics

Longevity for Chesapeake Bay blue crabs was estimated at 8 y and used in the modeling of growth, YPR, and life-table analysis of the mean age of the stock. Results of tagging studies conducted in Albemarle Sound, North Carolina (Fishler 1965), and in the Chesapeake Bay (McConaughy 1991) provided clear and compelling evidence that blue crabs attain an age of at least 6 y or 7.5–8 y, respectively, in the exploited stock. This finding sharply contrasted with current convention that blue crabs live a maximum of 3 y, even in the absence of fishing. The choice of the theoretical maximum age of 8 y was risk averse in terms of estimating natural mortality and threshold fishing mortality rates (Rugolo et al. 1997). The age invariant instantaneous rate of natural mortality (M) was 0.375.

Blue crab growth in carapace width at age was modeled using the formulation of von Bertalanffy (1938). The von Bertalanffy model parameters for both sexes combined were $CW_{\infty} = 262.5$ mm, $K = 0.59$ and $t_0 = 0.0115$ y (Fig. 29). Because age determination in blue crabs is not possible, we used modal carapace width size groups of 0–59 mm, 60–119 mm, and 120–79 mm to bound presumptive ages 0, 1, and 2, respectively. Carapace widths at age 3–7 were unspecified in the model; a point estimate for terminal width was set to 260 mm. Because the fitting procedure did not consider the distribution of width at age or width for ages 3–7, the precision of the model fit ($r^2 = 0.99$) was artificial.

A simple life table was developed to examine the impact of our finding of longevity of 8 y on the mean age and mean crab size in the stock under virgin conditions ($F = 0$) and for varying levels of fishing mortality (F). Our aim was principally to assess whether the modeled mean age and carapace width of the stock was consistent with the current convention that blue crab do not live longer than 3 y. The mean age of the *theoretical* stock, defined as all ages 0–8, was estimated under $F = 0$ and for levels of $Z = 0.8$ –1.5 (Table 5; Fig. 30). Under virgin conditions, the expected mean age of the theoretical stock would be 1.9 y with a corresponding mean CW of 179 mm. At levels of Z that exist in the contemporary blue crab fishery ($Z \approx 1.3$), the mean age of the theoretical stock would be approximately 0.7 y (87 mm CW). Comparison of these results is only meaningful against empirical data for which all sizes are fully represented. Because the age 0 cohort is characteristically not fully sampled by either the fishery or research surveys, we derived an alternative construct called the *observed* stock, consisting of all ages 1–8 (i.e., ≥ 60 mm CW). Blue crabs of this size are sufficiently large to be sampled by the fishery and research survey gear. Under virgin conditions, the expected mean age of the observed stock would be 2.8 y with a corresponding mean size of 211 mm CW (Table 5; Fig. 30). Under approximate current $Z \approx 1.3$, the expected mean age of the stock is 1.5 y (153 mm CW).

This analysis indicated that at levels of total mortality seen in the baywide blue crab fishery during the last four decades, 97.3% of all blue crabs in the observed stock and 98.6% of all crabs in the theoretical stock would be 3 y of age or less. The probability, therefore, of encountering a blue crab older than 3 y under contemporary fishing conditions is exceedingly small. These results are consistent with the view that blue crabs older than 3 y are rarely captured in the fishery or by research surveys, notwithstanding the fact that they cannot be aged. This would explain, or at least allow for the evolution of conventional wisdom that blue crabs do not *live* beyond 3 years of age. Our finding of longevity of 8 y for the Chesapeake Bay blue crab is not contradictory to the observed age or size structure of the stock. This life table analysis sufficiently reconciled the apparent disparity between conventional wisdom and the life-history characteristics adopted in this stock assessment.

Biological Reference Points

Threshold fishing mortality rates for the blue crab were estimated using yield per recruit and spawning stock abundance per recruit analysis. Thompson and Bell (1934) YPR analysis using life history parameters derived in this work provided the framework upon which to base a determination of the current stock status and fishery performance. Biological reference points associated with providing adequate spawners to maintain the reproductive potential of the stock and to prevent recruitment overfishing were emphasized. These included a series of F s, which resulted in a fixed %MSP ranging from 5–20%, encompassing levels suitable for this species. The threshold value of $F_{10\%}$ was selected for blue crabs based on biology and life history considerations. This assumption results in risk averse threshold levels of F : if blue crab life span is shorter, the %MSP needed to replenish the stock is lower, and the resulting threshold F would be significantly higher. Other factors were also considered in establishing threshold fishing mortality rates. The values of F_{MAX} and $F_{0.1}$ were calculated to provide reference points associated with maximizing YPR

rather than maintaining reproductive capacity. These reference points are considerably lower than those which maintain 10% of MSP and are generally associated with defining growth overfishing.

Data input to the YPR analysis included the distributions of length and weight at age, terminal length and age, schedules of maturity and partial recruitment, and natural mortality rate. Four maturity profiles were used in the analysis. These ranged from the most risk averse and biologically unrealistic schedule of lifetime spawning activity (flat-topped) to one that more closely reflects the current convention (Dome-3; Fig. 31). The latter was the most risk prone schedule considered. An intermediate spawning activity schedule was selected for the analysis (Dome-2), which is notably more risk averse than Dome-3, although slightly more risk prone than the flat-topped schedule.

Results of YPR modeling found that $F_{0.1} = 0.36$ and $F_{MAX} = 0.64$; whereas, that which provides for 10% of MSP ($F_{10\%}$) is 1.21 (Fig. 32). This latter value, corresponding to an annual exploitation rate of approximately 61%, was judged to be a valid threshold fishing mortality rate for the blue crab stock in comparison to other resources with similar life history characteristics. This decision was substantiated by results of a spawning stock abundance per recruit analysis (SSA/R) that estimated a replacement fishing mortality rate (F_{REP}) equal to 1.17 (Fig. 33). Other SSA/R reference points were $F_{HIGH} = 1.76$, suggestive of a level of fishing which could lead to stock collapse if maintained, and $F_{LOW} = 0.48$ which is similar in magnitude to $F_{0.1}$.

The fishing mortality rate (F) in 1996 was estimated at 0.865 ($\mu_{ABS} = 0.496$), approximately 35.2% greater than $F_{MAX} = 0.64$. All F s estimated in this study for 1956 to 1995 were below the threshold value $F_{10\%} = 1.21$. At fishing mortality from $F = 0.9$ – 1.0 , the %MSP in the stock ranged from 16.7–14.1%. For rates of F in excess of F_{MAX} , the stock is considered to be growth overfished. At current F , the theoretical YPR is approximately 2.0% less than that theorized under F_{MAX} . Hence, in theory, if the current fishing mortality rate was reduced by 26% to $F = 0.64$, we could expect a 2% increase in YPR and the cessation of growth overfishing.

In general terms, growth overfishing is related to the removal of animals from the stock at rates in excess of that which would maximize yield from each recruit. It results from a rate of fishing greater than which the losses in weight from total mortality exceed the gain in weight attributable to growth. The resulting length frequency of a growth-overfished stock is less broad than that which would be observed under virgin conditions. Excessive exploitation rates could result in what is termed *juvenescence* of the stock, characterized by a severe truncation in the length frequency at lengths above the minimum legal limit. Other applications of this concept of growth overfishing relate to the threshold level termed $F_{0.1}$, defined as the fishing mortality rate that corresponds to a point on the YPR function with a slope of 10% of that through the origin. Although the effect of fishing on future generations of the resource is one of the considerations behind $F_{0.1}$, the relationship is somewhat speculative.

Gear Saturation Analysis

A principal finding of this research was that despite a fivefold increase in total directed fishing effort since 1945 (Table 2; Fig. 13), fishing mortality has remained relatively constant since 1956 (Table 4). A near constant fraction of the stock has been removed

annually by fishing (Figs. 25, 26) since the mid-1950s irrespective of the levels of effort applied by the fishery. This long-term rise in effort coupled with relatively steady harvests (Fig. 14) has resulted in declining CPUE during the last four decades (Fig. 15).

Assuming constant q and stock size (Fig. 8), F would have been expected to rise steadily since the mid-1950s in proportion to the observed increase in f . We examined the lack of proportionality between F and f by empirical tests of q based on log-log regression analysis of q against f , and by linear regression analysis of F on f . If q was not constant in the bay blue crab fisheries, we assumed that the effect resulted largely from the saturation of static capture gear used as the primary means of harvest (Rugolo et al., this volume).

To test the hypothesis that q was inversely related to nominal effort, the value of q was related to f by log-log regression. Using estimates of $q = F/f$ derived from the VIMS trawl survey-based F s for 1956 to 1995 (Fig. 34) and from the Calvert Cliffs survey-based F s for 1968 to 1995 (Fig. 35), q exhibited a significant ($p < .0001$) inverse relationship to total directed fishing effort. The linear regression between F and f based on the VIMS survey F s (Fig. 36) revealed a significantly ($p < .007$) negative slope indicating gear saturation. The slope estimates from the linear models using the MDNR survey F s (Fig. 37) and the Calvert Cliffs survey F s (Fig. 38) did not differ significantly from zero. Because the slope estimates from these linear models were either negative or did not differ significantly from zero, these results suggest that fishing mortality on Chesapeake Bay blue crabs has been decoupled from effort over the range of f examined during 1956 to 1995 as a result of gear saturation in the pot and trotline fisheries.

Results of this analysis indicated that q has not remained constant since 1956. Rugolo et al. (1997) also found strikingly similar results of the nonproportionality between F and f in the New England American lobster fisheries, which have undergone a similar historical rise in f without a proportional rise in F . This phenomenon of rapidly declining CPUE coincident with an initial sharp rise in effort followed by relatively stable CPUE is also reported in other stationary gear-based fisheries, such as the longline skipjack tuna fishery of the Pacific Ocean and the South Australian rock lobster fishery. The nonproportionality between F and f is strong evidence that gear saturation is operating in the Bay's blue crab fisheries insofar as historical changes in stock availability or abundance, or in gear selectivity are not applicable. The contemporary decline in fishing performance described by commercial fishers is detectable. We conclude that it reflects a decline in the catchability of the gear rather than a decline in the status of the fully recruited stock as a result of gear saturation and gear competition. Catch per unit effort in the baywide combined blue crab fisheries has remained relatively constant since 1970 (Fig. 15).

The economic implications of this finding of gear saturation are profound. Because current fishing mortality on blue crab seems largely independent of changes in fishing effort over the range of effort examined, significant reductions in total effort would be required to realize marked increases in CPUE or in %MSP or to decrease F . At a minimum, a proportional change in these measures should not be expected by a specific reduction in directed effort. Significant improvements in these measures are unlikely to occur until total directed effort is approximately the level that existed in the mid-1950s. Current evidence suggests that the Chesapeake Bay blue crab fishing industry is severely overcapitalized in terms of the quantity of gear applied to the harvest. The combined fisheries are operating at extremely low levels of eco-

conomic efficiency, with a relatively constant harvest divided into increasing numbers of gear units.

CONCLUSIONS

In this research, blue crab vital rates and life history characteristics necessary to conduct an analytical stock assessment were described. In all instances where rates or parameters were estimated that would influence the assessment findings, and where empirical evidence couldn't guide selection among available options, we adopted a risk averse approach. Most notably, we used this approach in selection of the theoretical maximum age and spawning activity schedule. Individually, the selection of a maximum life span of 8 y or the lifetime spawning activity schedule described as Dome-2 operate to lower the threshold biological reference point $F_{10\%}$. Collectively, they result in a dramatically lower reference value than would be derived under conventional assumptions of longevity of 3 y and the rapid decline in spawning activity after first spawning. Formulating the assessment in this manner resulted in increased sensitivity to the finding of a stock demise and recruitment overfishing, because the gap between current F and the overfishing definition was narrowed.

Historical and contemporary fishery-independent research surveys spanning the last 5 decades indicated that current spawning stock biomass was at moderate levels as compared to historical maxima. The stock seemed to be maintaining total and spawning stock biomass at or near long-term averages measured since 1956. We found no evidence of a persistent stock decline. The decade of the 1980s was a period of above average abundance; the population has modulated to approximate long-term average abundance levels since that time. At the current fishing rate ($F \approx 0.9$ – 1.0), %MSP in the stock exceeds the 10% target level established for the stock. There is no evidence of a systematic or recent increase in fishing mortality to unprecedented levels. The combined baywide fisheries have exploited this resource at levels deemed appropriate for the species—that is, 30–45% for peeler/soft crab and 45–55% for hard crab. The stock is exhibiting dynamic equilibrium, with variable abundance as expected in an r -selected species. Recruitment has been variable since the 1950s and is presently at average levels.

Compelling evidence for the increase in stock abundance in the 1980s was demonstrated by the change in relative abundance in the exploitable stock measured by the Calvert Cliff fisheries-independent research survey and the commercial Potomac River landings. The VIMS and MDNR trawl survey programs also revealed above average exploitable stock abundance in the 1980s. The Chesapeake Bay blue crab stock seemed to be varying without trend during the last 5 decades, with an expected degree of interannual variability considering its short life span, life history characteristics, and reproductive strategy.

For the baywide commercial fishery, no contemporary (1970 to 1995) decline in CPUE has occurred following the initial exponential decline from 1947 to late-1960s. Baywide CPUE remained relatively stable thereafter. The stock is supporting the taking of near maximum sustainable yield in the long-term; no evidence of the effects of fishing on the ability of the stock to replace itself or to provide historical average yields was observed. There has been no demonstrable decrease in spawning stock biomass or in recruitment success. In customary stock assessment terminology, we judged the Chesapeake Bay blue crab stock to be *moderately to fully exploited at average levels of abundance*.

Fishing mortality rates and annual exploitation rates were estimated for 1956 to 1995. Relative exploitation has been noisy showing no persistent increases that would be consistent with overfishing. Absolute exploitation rates derived for the hard and peeler/soft components of the fishery ranged principally between 30–45% for peeler/soft crabs and 40–55% for hard crabs. Fishing mortality rates (F) on the combined stock have varied principally between 0.8–1.0 since 1956. Current fishing mortality rate was below the threshold reference level of $F_{10\%} = 1.21$ considered suitable for this fishery resource and has varied within approximately 20% of the long-term mean over the last 4 decades. The baywide stock has maintained both total stock and spawning stock biomass over the long-term. Because recent fishing mortality rates have been below the $F_{10\%}$ threshold, exploitation has not undermined total stock biomass. Current rates of fishing mortality have been supported by this stock for 40 y, indicating that the current level of fishing mortality is not detrimental to stock persistence. Our findings suggest that the fishery has been taking a fairly consistent fraction of the stock since 1956.

Given that the stock seems to be biologically stable, issues remain regarding the health of the fishery. The dramatic rise in directed effort since 1945, with the accompanying decline in fishing success, would normally be associated with an increase in fishing mortality rate. This was not demonstrated by the assessment. Catchability has varied with effort over the range of values observed from 1956 to 1995. This would imply that moderate reductions in fishing effort would likely not result in a proportional reduction in F because of the decoupling of F and f from nonconstant q . A possible explanation for the observed phenomenon of hyperdepletion in the blue crab fisheries relates to the primary capture gears. Crab pots and trotlines are static gears relying on the target organism to enter or hold on to the gear. They jointly account for approximately 90% of the baywide blue crab landings, and with the tremendous increase in gear fished for a finite resource, the effects of gear saturation and gear competition are evident.

We suggest that the blue crab fishery is severely overcapitalized in terms of fishing effort. As a result of gear saturation, marginal decreases in fishing effort would not realize proportional gains in either CPUE, %MSP, or YPR. The stock is strictly growth-overfished and, as a result of nonconstant q , substantial economic displacement would be required to decrease current F by 26% to achieve F_{MAX} . Increases in %MSP and YPR could be realized through size limit measures, along with regulating the taking of mature female crabs, without the considerable displacement costs associated with effort reductions alone.

We recommend that management be risk averse in its regulatory decision making. Management should maintain current fishing mortality rate below the threshold value of 1.21. This strategy should maintain yield under current stock levels and patterns of recruitment, absent environmental catastrophe. Management should be particularly averse to increases in fishing effort that would exacerbate current economic inefficiencies, or to permitting increases in gear efficiency (i.e., catchability). The latter recommendation acknowledges the rather delicate and poorly understood interplay between F and f in the contemporary blue crab fishery. Management should be proactive in its considerations, should stabilize and enhance the economic viability of the fishery, and should provide protection for the stock through maintenance of $\geq 10\%$ MSP. It should consider adopting strategies that increase yield per

recruit (e.g., size limit increases on both sexes) and spawning potential (e.g., limiting directed fisheries on mature female crabs).

Recommendations

In developing this assessment, we identified research and information needs that would enhance our understanding of the dynamics of the resource and its attendant fisheries. Rugolo et al. (1997) identify research that can be pursued or information that must be gathered to refine key component analyses of the assessment. We present here only a short list of essential areas for future research.

- (1) Develop a reliable and efficient method to age blue crabs.
- (2) Develop refined schedules of length at age and maturity at age given Item 1. An age-length key would enable devolution of the age structure from survey and fishery length frequency.
- (3) Stage harvest by length or age, thus allowing for the development of a catch at age matrix to be used in alternative assessment approaches.
- (4) Develop a baywide tagging study to provide information on growth, natural mortality, longevity, fishing mortality, size selectivity, catchability, reporting rates, and harvest distribution.
- (5) Estimate catchability by size category and sex for all principal gear types in the fishery.

- (6) Estimate recreational fishing effort, participation, and harvest for the baywide recreational fishery.
- (7) For all segments of the baywide fishery, improve information on the magnitude of the catch, directed effort, discard losses, and the biological characterization of the catch (particularly the peeler/soft crab fishery). Estimate under-reporting inherent in commercial and recreational harvest data.

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Erratum

Rugolo, L. J., Knotts, K. S., Lange, A. M. (1998) Historical profile of the Chesapeake Bay Blue Crab (*Callinectes sapidus* Rathbun) fishery. *J. Shellfish Res.* 17(2):383–394.

The author would like to make the following corrections:

- p. 1, Abstract section:
 - line 12, 38% should read 47%
 - line 13, should read "... Scrapes and pound nets contributed <1% of the total since 1981."
 - line 15, 10 should read 13
 - line 16, 19 should read 20; 1984 should read 1985; '21 to 35' should read '10 to 17'
 - line 17, 'increase in 1945' should read 'increase since 1945'
- p. 1, column 1, line 18, 'most commercial' should read 'most important commercial'
- p. 2, column 1, line 35, 1980s should read 1980
 - column 2, line 6, were should read are
 - line 19, fisheries should read fishers
 - line 31, 20% should read 25%
- p. 6, column 2, the word 'blue' should be deleted from the first line of the Figure 4 legend.
- p. 7, Figure 5 legend, line 2 should read '10⁶ kg, and 127+ mm carapace width Calvert'
 - line 3, delete 'the time periods'
- column 1, line 10, 10.5 should read 12.9
 - line 11, 19.1 should read 19.6, 1984 should read 1985
 - line 13, 9.5 to 15.9 should read 10.2 to 17.1
- column 2, line 21, 38% should read 47%
 - line 36, should read, "resolved to accurately reflect effective effort in the fishery."
- p. 10, column 1, line 31, pound should be kg
 - line 32, pound should be kg
- column 2, line 14, post should be pose
- p. 11, column 1, line 5, 10 to 19 should be 12.9 to 19.6
 - line 6, 1984 should be 1985, 10 should be 10.2
 - line 7, 21 to 35 should be 10.2 to 17.1
- column 2, line 16, 1982 and 1992 should be 1982 to 1992

TABLE 4.

Commercial harvest (10⁶ kg) of female blue crabs baywide and in Maryland; harvest (10⁶ kg) of mixed market category in Maryland; and CPUE in the Maryland pot (mean kg/pot/mo) and trotline (mean kg/trotline-h/mo) fisheries.

Year	Maryland				
	Baywide Female	Female	Mixed	Pot CPUE	Trotline CPUE
1982	5.57	4.99	4.99	12.89	10.20
1983	10.16	6.35	4.08	14.89	13.35
1984	8.48	6.35	3.63	16.41	16.08
1985	10.29	8.16	3.63	19.59	17.05
1986	6.34	5.44	3.17	16.58	13.62
1987	6.44	4.99	2.27	13.86	14.03
1988	8.98	5.44	2.72	14.18	13.10
1989	7.75	4.99	3.63	12.99	13.43
1990	9.12	5.44	3.63	15.90	14.74
1991	5.62	5.44	3.17	15.45	16.40
1992	4.76	4.54	1.81	10.65	11.15
1993	10.20	8.62	3.17	13.11	14.39
1994	7.17	7.71	1.81	10.24	12.17

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COVER PHOTO: "Old Blue Eyes" *Argopecten irradians irradians*, by Scott Hughes of BioLums Photography

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Proceedings of a Symposium on

**GENE CONSERVATION:
MANAGEMENT AND EVOLUTIONARY UNITS
IN
FRESHWATER BIVALVE MANAGEMENT**

Held in Conjunction with the Annual Meeting of the
National Shellfisheries Association
89th Annual Meeting

at
Fort Walton Beach, Florida
April 19-20, 1997

GUEST EDITOR
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Lampsilis sp. attempting to attract a fish host. The mantle flap, which appears to imitate a small fish, is postulated to have evolved as a mechanism to attract fish. For many freshwater bivalves, a host fish is necessary for the attachment, growth, and development of the parasitic glochidial life stage. This *Lampsilis* specimen was photographed in the Elk River, West Virginia by Craig Stihler, West Virginia Division of Natural Resources.

GENE CONSERVATION: MANAGEMENT AND EVOLUTIONARY UNITS IN FRESHWATER BIVALVE MANAGEMENT—INTRODUCTION TO THE PROCEEDINGS

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Due to the consequences of human population expansion, species are increasingly being lost and the speciation process, which creates future biodiversity, is being severely constrained by habitat loss (Erwin 1991). Perhaps no faunal group illustrates this trend more profoundly than freshwater bivalves of the family Unionidae. North America possesses the world's greatest diversity of freshwater bivalves (commonly referred to as freshwater mussels). However, this biodiversity legacy is in jeopardy of being lost with more than 70% of recognized species endangered, threatened, or of special concern (Williams et al. 1993). The principal threats to these bivalves are pollution, loss or alteration of habitat, loss of obligately parasitized hosts that support unionid larvae during their early development, and the aggressive expansion of the exotic mussel *Dreissena polymorpha* (Williams et al. 1993). Consequently, freshwater bivalves need immediate conservation efforts to identify and sustain existing biodiversity. However, these animals present significant challenges to conservation biologists. They show broad taxonomic uncertainty by virtue of phenotypic plasticity, complex life histories, and varied modes of reproduction. To address basic conservation challenges for planning and implementing biologically sound management programs, a thorough understanding of the evolutionary relationships among all levels of freshwater bivalve biodiversity is essential.

The most fundamental constituent of biodiversity is genetic diversity (Meffe and Carroll 1994). It is well established that detectable genetic variation is distributed hierarchically from phylogenetic variation among reproductively isolated taxa to geographic variation within species (Avice 1994). The identification and preservation of genetic diversity is increasingly being accepted as the foundation for contemporary conservation strategy.

Effective conservation programs require clearly definable units of management. Molecular genetics has recently achieved an important place in contemporary conservation biology as it has proven to be a robust tool for identifying reproductive isolation among populations. Consequently, genetic information offers an objective means of delineating management units, and provides an evolutionary framework from which to develop and evaluate conservation priorities. However, attempts to identify conservation units within species often require the imposition of divisions on an evolutionary continuum, a concept that has always evoked controversy (Moritz et al. 1995). Intense debates are currently being waged in conservation circles surrounding species concepts (Baum 1992, Wayne 1992, Mayden and Wood 1995) and the correct interpretation and implementation of "distinct population segments" as intended by the framers of the Endangered Species Act (Moritz 1994, Moritz et al. 1995, Vogler and DeSalle 1994, Nielsen 1995, Waples 1995, Pennock and Dimmick 1997, Waples

1998). In an attempt to define distinct population segments of Pacific salmon in terms that would allow the preservation of genetic variability, the National Marine Fisheries Service adopted the evolutionarily significant unit (ESU) concept (Waples 1991) originally proposed as a conservation tool in zoos by Ryder (1986). An ESU is a population or aggregate of populations that (1) is substantially reproductively isolated from other conspecific groups, and (2) represents an important component in the evolutionary legacy of the species (Waples 1991). Many molecular systematists disagree with the ESU interpretation of distinct population segment and instead argue that documentation of deep evolutionary separation should be evidence of distinct species (see synthesis by Mayden and Wood 1995). A significant element in the aforementioned debate is the role that molecular genetic data can play in resolving the delineation of ESUs and/or species (see Dizon et al. 1995, Moritz et al. 1995, Mulvey et al. 1997, Lydeard and Roe 1998, Roe and Lydeard 1998).

The need for urgent conservation measures and for the establishment of guidelines for delimiting conservation units in freshwater bivalves prompted an invited symposium titled "Gene Conservation: Management and Evolutionary Units in Freshwater Bivalve Management" held in association with the 89th Annual Meeting of the National Shellfisheries Association, Fort Walton Beach, Florida, in April 1997. This symposium brought together conservation geneticists to discuss empirical and theoretical insights into identifying evolutionarily significant lineages and to have these perspectives enlightened by resource agency personnel as to the roles of science and government in developing policies to conserve freshwater bivalve diversity. The nine papers included in these proceedings discuss three broad issues relative to freshwater bivalves: (1) guidelines and methodology for delineation of conservation units; (2) the state of the science in population genetics and molecular systematics, and (3) the role of emerging genetics issues in freshwater bivalve conservation. The proceedings contain contributions from all but three of the participants in the Fort Walton Beach symposium; time limitations and other obligations prevented Marsha Black, Jim Williams, and Ren Lohoefer from providing written papers. Their contributions, including a round-table discussion at the end of the symposium, were highly valued nonetheless.

In his keynote address to the symposium, Dr. Brian Bowen presented an insightful synopsis of issues regarding the recognition of evolutionarily critical lineages for conservation, providing examples from the application of his own research on marine turtles and sturgeons. His contribution to these proceedings is a short opinion piece that outlines an intriguing perspective on defining units of conservation. Bowen uses these proceedings to propose a

new conservation category, Geminate Evolutionary Units (GEUs), intended to delineate new evolutionary pathways and ultimately provide a mechanism for recognition of evolutionary potential.

In their contribution to the proceedings, Kevin Roe and Charles Lydeard take issue with the Evolutionarily Significant Unit (ESU) concept. The authors provide DNA-based molecular variation obtained within and among species in the genus *Potamilius* as a case study to convincingly argue that the ESU concept overlaps sufficiently with contemporary species concepts such that these evolutionarily divergent assemblages could also qualify as distinct species. Roe and Lydeard's conclusions appear to confirm the assertion by Moritz (1994) that certain ESUs "complement rather than replace species" designations. The authors pose ESU criteria that would limit its designation to the intra-specific level, for which it was initially intended.

An extensive survey of population genetic structure is one prerequisite to identifying evolutionarily important lineages. Dave Berg et al. contribute their findings from a thorough population genetics survey for the wide-ranging species *Quadrula quadrula*. In this study, which examines allozyme variation, Berg et al. found evidence for high levels of gene flow among geographic populations from the Ohio and Mississippi River drainages. They conclude that large river habitats may be more stable, capable of supporting larger populations of bivalves, and may contain fishes with greater dispersal capability than small streams. Berg et al. suggest that conservation efforts for wide ranging species (e.g. *Q. quadrula*) inhabiting large rivers should be directed to protect large population units that encompass the breadth of genetic variability.

Ron Johnson and three co-authors compared levels of genetic diversity and physiological response in populations of four bivalve species in the Cache and White Rivers of Arkansas. Johnson et al. identified heterozygote deficiencies at all 15 allozyme loci surveyed. The authors considered numerous explanations for this deficiency including bottleneck effects, the Wahlund effect, null alleles, linkage disequilibrium, inbreeding, and selection. The authors also investigated cellulolytic (i.e., cellulase) activity within populations and species as a function of heterozygosity, testing the theoretical concept that increased multiple locus heterozygosity results in increased fitness.

Certain freshwater bivalve species are hermaphroditic with the potential for simultaneous gonadal development and subsequent self-fertilization (Van Der Schalie 1966, Kat 1983). A simultaneous hermaphroditic mode of reproduction can profoundly impact the genetic population structure of freshwater bivalves but has remained undocumented. Walter "Randy" Hoeh and co-authors tackled this very complex issue in hermaphroditic freshwater bivalves of the genus *Utterbackia*. Hoeh et al. demonstrated a high degree of self-fertilization in hermaphroditic *Utterbackia*. Rates of cross-fertilization and selfing varied among populations of some species. The authors provide convincing evidence that the large differences in genetic structure between *Utterbackia* species with different modes of reproduction are due to the effects of severe inbreeding alone or in conjunction with genetic drift. Hoeh et al. conclude that the mating system and distribution of genetic variation in bivalve populations should be identified as prerequisites to conservation initiatives. Conservation of these species may require maintenance and recovery of a number of small populations.

Margaret Mulvey, Hsiu-Ping Liu, and Karen Kandl each contributed presentations to the symposium describing molecular phylogenetic relationships of the bivalve taxa *Elliptio*, Anodontinae, and *Pleurobema*, respectively. They combined their presentations,

at the request of the editors, to provide a comprehensive overview of contemporary molecular genetic methodologies and their application to the conservation of freshwater bivalves. This contribution is designed to provide a resource for future researchers interested in conservation genetics of freshwater bivalves. Mulvey et al. cover the entire range of techniques from protein electrophoresis to DNA-based technologies. The authors provide the established sequences of oligonucleotide primers for polymerase chain reaction amplification of three mitochondrial DNA regions and two transcribed spacer regions of nuclear ribosomal DNA. These primers should be applicable in most freshwater bivalve molecular systematics investigations. The paper provides insight into the potential conservation applications for these various techniques including population genetics, molecular systematics, bivalve-host relationships, and forensics. In addition, Mulvey et al. provide examples from their own work with member species of Ambloinae and Anodontinae.

How can genetics inform and assist management decisions regarding whether or not a species should be listed under the Endangered Species Act or its State equivalents, or recover a species already under State or Federal protection? Recovery of depleted species or relict populations demands identification and conservation of as much of their genetic variability as still exists. Rita Vilella et al. discuss the importance of maintaining genetic diversity within and among populations and the potential for spread of pathogens and parasites through conservation efforts like relocation. They suggest approaches that could lead to improved success of relocations, with the ultimate goal of maintaining the evolutionary lineages of freshwater bivalves. In addition, Vilella et al. (along with Roe and Lydeard and Mulvey et al.) place emphasis on the importance of recognizing the bivalve-host relationship in management decisions inasmuch as gene flow among populations of bivalves is dependent upon the obligately parasitized host.

Managers are faced with escalating bivalve declines and are accountable for applying the Endangered Species Act to counteract them. Representatives of the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) were active participants in the symposium and the proceedings. Susi von Oettingen presented "A National Strategy for the Conservation of Native Freshwater Mussels" that has been developed by a joint State and Federal National Native Mussel Conservation Committee. This document is reproduced here in its entirety.

In an effort to enlighten participants on contemporary guidelines for delineating units of management, Marta Nammack presented a review of the history and use of the ESU concept as it was applied to Pacific salmonids. It is noteworthy, however, that freshwater bivalves and other invertebrates are not included in legal definitions of distinct population segments or ESUs. In the absence of ESA legislation to protect intraspecific groups of freshwater bivalves, significant portions of the evolutionary legacy of some species may be lost.

We believe this symposium will heighten awareness of the fact that effective freshwater bivalve conservation efforts must include maintenance of genetic variation because of its consequent effect on evolutionary potential, and ultimately, long-term survival of species. Vilella et al. maintain that the preservation of genetic diversity is a prerequisite to the conservation of ecological and evolutionary processes. Mulvey et al. ask "In the face of huge ecological and demographic issues in the conservation of freshwater bivalves, does it make sense to spend limited resources on genetic studies?" Given declining populations and extensive biological and ecological information gaps, genetic information is

crucial to making difficult decisions on conservation. The studies presented here cover the tools, techniques, issues, and interpreta-

tions to allow the reader to answer that question to his or her own satisfaction.

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WHAT IS WRONG WITH ESUs?: THE GAP BETWEEN EVOLUTIONARY THEORY AND CONSERVATION PRINCIPLES

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KEY WORDS: Conservation genetics, evolution, speciation, wildlife management

INTRODUCTION

When biological information is translated into conservation policy, scientists are challenged to transform previously nebulous concepts into quantifiable terms. A prominent example of this process is the need to define the units of wildlife protection in a legal context, rekindling a debate over species concepts and prompting several innovative solutions (Cracraft 1983, Ryder 1986, Avise & Ball 1990, Vane-Wright et al. 1991, Vogler & DeSalle 1992, Wayne 1992, Waples 1995). Prominent among these solutions are the twin concepts of management units (MUs) and evolutionarily significant units (ESUs).

MUs are recognized as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles (Moritz 1994). Seven decades of theoretical population genetics demonstrate that differences in allele frequencies denote populations with independent demographic trajectories (Fisher 1930, Wright 1931, Kimura 1964, Slatkin 1987), and empirical studies confirm this expectation under most circumstances (Avise 1994). Hence MUs are well grounded in principles of population biology. MUs are valuable in conservation because they describe the fundamental units of wildlife management: reproductively isolated populations.

The ESU is intended as a category above management units, describing a population or groups of populations that may comprise a novel evolutionary trajectory, as indicated by long-term isolation or significant divergence in ecological, morphological, or genetic traits (see Waples 1991). The ESU concept is valuable because it allows conservationists to circumvent sticky questions about taxonomy and species concepts, and focus on the preservation of major evolutionary subdivisions. However, several recent reports have expressed dissatisfaction with the ESU, stemming in part from the recognition that some faunas that probably merit conservation resources do not qualify under current definitions (see Barlow 1995; Stauffer et al. 1995, Pennock & Dimmick 1997, Karl & Bowen 1999). Hence a quandary exists that may be attributed in part to an incomplete body of evolutionary theory. Though MUs can be defined on a solid foundation of population genetic theory, exemplified by the Wright-Fisher model of genetic drift, there is no corresponding model that unifies the search for evolutionary units. In applying the principle of ESUs, conservationists probe one of the darkest chasms in evolutionary biology, embodied by the question "How do new organisms arise?" There is no consensus on how isolation, selection, founder events, drift, hybridization, recombination, and mutation fit together to produce evolutionary novelty, and so the conceptual bridge between evolutionary processes and conservation is incomplete. If no consensus exists on how organismal evolution occurs, how then can we define evolutionarily significant units with confidence?

ESU DEFINITIONS: SEEING DOUBLE BUT FEELING SINGLE

Perhaps the mostly widely used ESU definition, the NMFS interpretation of the U.S. Endangered Species Act, includes two criteria. An ESU is a population (or group of populations) that (1) is substantially reproductively isolated from other conspecific populations, and (2) represents an important component in the evolutionary legacy of the species (Waples 1991). The first half of this definition can be defined and defended on the basis of separations possibly followed by reproductive incompatibility (allopatric speciation) (Mayr 1982). The second component is more difficult to delineate, and for this reason it received further clarification in a recent treatise on ESUs: The evolutionary legacy of a species is the genetic variability that is a product of past evolutionary events and that represents the reservoir upon which future evolutionary potential depends (Waples 1995).

As in the NMFS interpretation of ESUs, a dichotomy is apparent in the definition of evolutionary legacy (Waples 1995): *past evolutionary events* and *future evolutionary potential*. The first category is the realm of phylogenetic systematics, in which organismal lineages generated by past evolutionary events are identified by morphological and genetic criteria. Wildlife managers can use the depth of these evolutionary separations as a yardstick to assign conservation priorities (Avise 1989). The second component is more difficult to identify: the progenitors of future evolutionary diversity. To accomplish this would potentially resolve the question of how new species arise. Much progress has been made on this front in recent decades (Eldredge & Gould 1972, Carson & Templeton 1984, Barton & Charlesworth 1984, Otte & Endler 1989, Giddings et al. 1989, Palumbi 1994, Veron 1995, Schluter 1996, Smith et al. 1997, Klicka & Zink 1997), but few would argue that speciation processes are fully understood.

Because the progenitors of future species are so difficult to identify, the ESU criterion of "future evolutionary potential" is not often invoked, and the primary emphasis in the resolution of ESUs has been on phylogenetic criteria and the preservation of populations that show evidence of long-term isolation. This is illustrated in a genetic context by the ESU definition proposed by Moritz (1994): "ESUs should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci." The considerable value of this definition is in providing objective criteria for the level of divergence in neutral genetic markers that may characterize sibling species. (The parallel morphological criterion may be a fixed difference for at least one morphological character.) This definition is compatible with the first half of Waples (1995) definition of evolutionary legacy (the products of past evolutionary events), but does not address the second half (the progenitors of future biodiversity).

GEMINATE EVOLUTIONARY UNITS

The difficulty in identifying future evolutionary potential does not reduce the importance of this phenomenon in conservation and wildlife management. As noted by Balon (1993) "the maintenance of biodiversity is not a static phenomenon but a process in which creative forces are as important as destructive ones." Though the criteria for geminate species are elusive, it is very likely that isolation and gradual genetic divergence are not the only routes towards speciation. Hence the future evolutionary potential may include the phylogenetic criteria outlined by Moritz (1994, see also Bernatchez 1995, Moritz et al. 1995), but likely encompass other phenomena as well. Viewed from this perspective, future evolutionary potential may represent a third conservation category, distinct from MUs that are defined by population-level distinctions, and ESUs that are defined primarily by phylogenetic distinctions.

In terms of conservation goals, one does not want to hasten the extinction of evolutionary lineages, nor does one wish to prevent their arrival. In order to appreciate both ends of this evolutionary treadmill, the definition of evolutionary legacy by Waples (1995) may be divided into two components. The first component, evolutionary distinctiveness, is essentially concordant with Moritz's (1994) definition, and by precedent should retain the name *Evolutionary Significant Unit* (ESUs). The second component, the progenitors of future biodiversity, may be distinguished as *Geminate Evolutionary Units* (GEUs). This distinction is based on the recognition that divergence through long-term isolation is not the only route to speciation. Hence the "phylogenetic" ESU defined by reciprocal monophyly (Moritz 1994) does not incorporate all sources of evolutionary novelty.

How do we identify geminate species, or future evolutionary potential? Not easily, with current technology and knowledge, but the fact that we cannot readily resolve geminate species does not mean that this goal will never come to pass. For example, researchers have long suspected that regulatory operons may catalyze evolutionary processes including speciation (Britten & Davidson 1969, Kriebler & Rose 1986, McDonald 1990), but only in recent years have these regulatory sequences become accessible. It is possible that a thorough understanding of such genetic elements will allow greater precision in identifying potential evolutionary novelties. This is a challenge for the next generation of researchers, but clues already exist that may indicate future evolutionary potential.

One simple criterion draws on the vicariance school of biogeography (see Nelson & Platnick 1981). A population that has been isolated from other populations in recent geological time, and is likely to remain isolated for an extended geological period, must be considered a GEU. Consider the marine species that were initially isolated by the Isthmus of Panama into West Atlantic and East Pacific cohorts. Taxon pairs across this barrier probably all qualify as ESUs at present, but they almost certainly did not approach this category at the same pace (Lessios 1981, Knowlton et al. 1993). In contrast, they all became GEUs during the same geological moment, around the interval where water exchange ceased between Pacific and Atlantic. Other examples may include the freshwater taxa that have recently invaded higher latitudes in response to glacial retreats (see Bernatchez & Wilson 1998 and references therein).

A second criterion may be the behavioral barriers to gene flow reported from closely related morphotypes or "races" of fish (Barlow 1995, Stauffer et al. 1995). These types can be genetically

indistinguishable (or distinguishable only at the level of MUs) but separated by substantial behavioral barriers such as homing to different drainages or spawning at different times of the year. This type of evolutionary trajectory could potentially lead to speciation.

A third criterion may be niche expansion, in which a subpopulation invades a new habitat adjacent to the parent population. In this process of *ecological speciation*, strong selection to adapt to the new habitat may induce speciation, even with a residual level of gene flow from the ancestral population (Schluter 1996, Smith et al. 1997).

A fourth criterion may involve polytypic species characterized by high diversity in morphological, ecological, or genetic traits. Based on evolutionary theory, several researchers have argued that conservation priorities should focus on preservation of diversity which allows biota to adapt and produce new evolutionary lineages (Frankel 1974; but see Lynch 1995, Lande & Shannon 1996). This concept can be extended to speciose genera that, on the basis of recent track record, may also be well-springs of future biodiversity (Erwin 1991).

A fifth criterion, and perhaps the most important one, is morphological differentiation coupled with homogeneity in conventional surveys of neutral genetic markers. (Notably, the reciprocal condition, genetic distinctiveness coupled with morphological homogeneity, readily fits into the phylogenetic ESU defined by Moritz, 1994). This GEU category includes chromosomal rearrangements and duplications, by which new species can arise virtually overnight. Lewis (1980) estimated that 70–80% of angiosperm plant species have polyploid origins, and such speciation phenomena have been documented in animals as well (Sites & Moritz 1987). Polyploidization events may be relatively common in freshwater mussels (Park and Burch 1995) but whether these duplications are a catalyst for speciation is unclear.

Where do GEUs sit in the hierarchy of MUs and ESUs? A theme common to the five categories listed above is that conventional genetic assays such as protein electrophoresis and DNA sequence comparisons may not distinguish GEUs, or may distinguish them at a level typical of MUs. Not all geminate species have existed for sufficient time to allow differentiation in terms of neutral genetic markers (see box 7.3 in Avise 1994). Hence, in some circumstances the genetic criteria for defining GEUs may be exactly the opposite of those for defining ESUs. In this case biologists must recognize that a lack of genetic distinctiveness at neutral loci, in the face of morphological, behavioral, or biogeographic differentiation, may denote an important finding.

CONSERVATION POLICY: MANAGEMENT OF BIODIVERSITY IN FRESHWATER MUSSELS

The point of this essay is that geminate species are underappreciated in the context of evolutionary guidelines for conservation, partly because the relevant body of evolutionary theory is incomplete. Long-term isolation of populations is not the only generator of new species, and conservation policy may benefit from this recognition. To emphasize the distinction between divergence and evolutionary potential, I suggest the label "Geminate Evolutionary Unit" for groups that show evidence of the latter phenomenon.

How can the distinction between ESUs and GEUs influence management policies such as those implemented under the U.S. Endangered Species Act? Recognition of GEUs should not substantially alter the current criteria for legal protection, because the

ESU interpretation employed by U.S. wildlife management agencies already contains essential elements of the GEU (Waples 1995). Since ESA policy already affords protection to evolutionary units below the species level, there seems to be sufficient flexibility to protect GEUs as well (Waples 1998). The value of the GEU designation is not in reformulating public policy, but in prompting the recognition of two distinct motivations underlying the current ESU category: the husbandry of divergent evolutionary lineages and the propagation of new ones.

Freshwater mussels may provide many examples of the distinction between ESUs and GEUs because of the documented discordance between morphological and molecular data sets in bivalve systematics. Cases in which suspected morphological divergence between populations is paired with genetic homogeneity, and cases in which genetic divergence is paired with morphological uniformity, are well documented (Hoeh 1990, Mulvey et al. 1997, Roe & Lydeard 1998).

In recent years, dire population trends, habitat degradation, and invasion of exotics have prompted strong conservation concerns for freshwater mussels of North America (Williams et al. 1993), and this in turn has rekindled interest in the taxonomy and systematics of the two North American mussel families, Unionidae and Margaritiferidae. The relationships among many species and genera are controversial (Lydeard et al. 1996), and cryptic species may be common because of the famous difficulties in resolving mussel relationships with soft anatomy and conchology (Johnson 1970, Davis 1983, Hoeh 1990). Given the difficulties with making phylogenetic inference from mussel morphology, investigators may increasingly rely on molecular and biochemical evaluations to resolve controversial taxonomy. Though the value of genetic assays is beyond debate, there is a possibility that species or subspecies designations based on morphological, behavioral, ecological, or biogeographical evidence could be discounted in favor of molecular genetic evaluations. Such streamlined decision making would be a disservice to conservation and taxonomy. Researchers must bear in mind that genetic homogeneity, when coupled with differentiation in other meaningful traits, may signal an important evolutionary phenomenon. Davis (1983), Roe & Lydeard (1998), and others anticipate this consideration by recommending morpho-

logical and distributional studies as a necessary companion to molecular genetic surveys.

CONCLUSION

The MU fits readily into the framework of population biology and wildlife management [although not without limitations (Dizon et al. 1995)], and the ESU is largely based on phylogenetic principles such as reciprocal monophyly. These two categories represent a logical progression from shallow population separations to species and genera-level divisions. However, current ESU definitions encompass not only the products of past evolutionary separations, but the progenitors of future evolutionary lineages. The latter group does not arise exclusively from the former, but may also be the product of hybridization, polyploidization, founder events, and other reticulated evolutionary processes. Since evolutionary novelty does not arise exclusively from the progression of MUs towards ESUs, wildlife managers should distinguish ESUs in the phylogenetic sense from evolutionary novelties and possible geminate species.

Much of conservation is intended to prevent the premature departure of species, but human activities almost certainly curtail the arrival of new species as well. To make this distinction clear, the GEU is proposed as a conservation category for populations that may represent new evolutionary pathways. Such populations are identified in some cases by morphological, behavioral, or ecological differentiation in the face of genetic homogeneity. Other distinguishing features await discovery. The GEU may be the most difficult category to define, but this should not deter a recognition of the importance of evolutionary novelty in conservation.

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SPECIES DELINEATION AND THE IDENTIFICATION OF EVOLUTIONARILY SIGNIFICANT UNITS: LESSONS FROM THE FRESHWATER MUSSEL GENUS *POTAMILUS* (BIVALVIA: UNIONIDAE)

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ABSTRACT Accurate identification of biological entities is critical to the timely and efficient preservation of biodiversity. Concepts that define segments of biological diversity—species and evolutionarily significant units (ESUs)—should reflect our current knowledge of the biological world. Conflation of different hierarchical definitions of taxa has the potential to obscure distinct biological entities in need of protection. The concept of the ESU has been criticized because it includes within its definition distinct biological entities that otherwise would be recognized as species. Herein we evaluate several versions of the evolutionary significant unit concept and provide as a case study an analysis of geographic variation of a species of freshwater mussel, the inflated heelsplitter *Potamilus inflatus*. We demonstrate that as currently formulated, the ESU overlaps considerably with many biological definitions of species and therefore includes, in addition to distinctive populations, entities that would be recognized as species under many contemporary species concepts. Conflation of these two hierarchically distinct entities results in the ambiguous application of these concepts and inaccurate estimations of biological diversity. Continued use of the ESU concept has ramifications for the protection of invertebrate taxa under the Endangered Species Act of 1973 as amended in 1978. Recommendations for modification of the ESU concept are presented.

KEY WORDS: species, evolutionarily significant units, *Potamilus*, freshwater mussels, unionids, cytochrome oxidase I

INTRODUCTION

Accurate identification of biological diversity is considered critical to its conservation. As stated by Mayden and Wood (1995) if "... the ability to identify diversity correctly is impaired, then ... our efforts to conserve and understand these entities further will be ineffective." Species play a prominent role in biological sciences from ecological studies to molecular systematics. Unfortunately, our ability to perceive differences that exist between species often exceeds our ability to accurately define such entities. Since the term "species" has become part of the scientific vocabulary, its definition has changed from typological, to nominalistic, to the contemporary definitions in use today. A typological definition requires that species are relatively static entities and rejects the variation known to occur in natural populations. In a nominalistic definition, species are artificial constructs created by humans and only the individual organism is real. Alternatively, contemporary definitions recognize the variation that is known to exist and that historical ancestor-descendant relationships exist among individuals as well as higher taxa (species).

The purpose of this paper is to evaluate several concepts of the evolutionary significant unit (ESU) with regard to the degree to which they overlap conceptually with existing definitions of species. Herein, we compare the entities defined under the ESU concept to those defined under several contemporary species concepts. We will demonstrate that as currently formulated, the ESU overlaps considerably with many biological definitions of species and therefore includes, in addition to distinctive populations of species, entities that would normally be recognized as species. Current federal law restricts the protection of ESUs to vertebrate populations under the Endangered Species Act of 1973 as amended in 1978 (Public Law 95-632 (1978), 92 Stat. 3751), and we feel that the use of the freshwater mussel *Potamilus inflatus* as a case study highlights another major shortcoming of the ESU concept: the exclusion of invertebrate taxa from legal protection.

Many species concepts have been proposed over the past 50

years. They differ from earlier versions in that they include, to a greater or lesser degree, a historical component that more accurately reflects our current knowledge of the natural world. These concepts include, but are not limited to, the biological species concept (BSC) (Mayr and Ashlock 1991), the phylogenetic species concept (PSC) (Cracraft 1983, Wheeler and Platnick 1997), the recognition species concept (RSC) (Paterson 1993), and the evolutionary species concept (ESC) (Simpson 1961, Wiley 1978). The various criteria for each of these concepts are presented in Table 1. It is not the purpose of this paper to evaluate the various species concepts included here, as that has been done more thoroughly elsewhere (e.g., Wiley 1981, Mayden and Wood 1995, Mayden 1997), but rather we propose for the purpose of this study that entities satisfying most (or all) of the above concepts should be considered species, both biologically and legally.

Whereas the ESA is understood to be a legal document and not a conceptual definition of species, it includes within its definition of "species" those entities (populations) that do not conform to any of the contemporary biological definitions of species that are in use today. In addition to biological species, the ESA includes "... any subspecies of fish or wildlife or plants, and any *distinct population* segment of any species of *vertebrate* fish or wildlife which interbreeds when mature" (emphasis ours).

Ryder (1986) was the first to use the term "ESU," "[o]ut of a sense of frustration with the limitations of current mammalian taxonomy in determining which named subspecies actually represent significant adaptive variation." According to Ryder (1986), ESUs represent "subsets of the more inclusive entity species, which possess genetic attributes significant for the present and future generations of the species in question." Waples (1991) noted that whereas the ESA allowed listing of distinct vertebrate populations as "species" it gave no guidelines on how population distinctiveness was to be evaluated. In an effort to clarify species determination for populations under the ESA, Waples defined a vertebrate population to be distinct and therefore a species under

the ESA if "... the population represents an *evolutionarily significant unit* of the biological species" (emphasis ours). The term "ESU" was defined by Waples as a population (or group of populations) that (1) is substantially reproductively isolated from other conspecific population units, and (2) represents an important component in the evolutionary legacy of the species.

The ESU concept was conceived as a replacement for the class "subspecies" by Ryder (1986) because of problems associated with the application of that concept (see also Cracraft 1992). Since Waples formalized ESUs as subspecific entities, several other definitions have appeared (Moritz 1994, Vogler and DeSalle 1994). Each of these definitions increases the diagnosability of ESUs by more clearly delineating what an ESU is, however, a conceptual problem arises because all of the definitions proposed for ESUs are virtually identical to preexisting definitions for species. This fact has been recognized by several authors (Moritz 1994, Vogler and DeSalle 1994, Mayden and Wood 1995), although only Mayden and Wood saw this development as problematic. The problem is that there are currently two conceptual entities: ESUs and species, the former a subset of the latter, yet both are defined using the same criteria. Ultimately, in order to allow for the accurate delineation and protection of biological diversity, we feel that biologically sound and unambiguous definitions of biological entities must serve as the basis for the recognition of species and other taxa both conceptually and legally.

Examination of the various ESU and species concepts (Table 1) will serve to more clearly illustrate this point. A comparison of the ESU concept of Waples (1991) and the biological species concept of Mayr and Ashlock (1991) reveals a great similarity, and the crux of the problem. Both concepts rely primarily on the concept of reproductive isolation and because both are based on the same criteria, it seems inevitable that they would also identify the same biological entities. An argument could be made that the words "conspecific" and "... of the species" serve to distinguish Waples' ESUs from species. However, what criteria do we use to determine what a species is? If reproductive isolation is used to distinguish between species, as is required by the BSC, can we also use it to separate ESUs within that species? Moritz's (1994) attempt to more rigorously define the ESU concept succeeded in perhaps

increasing the diagnosability of ESUs, but did not correct the overlap with contemporary species concepts. The reliance of the ESU concept of Moritz on reciprocal monophyly is the source of the conceptual ambiguity. By definition, taxa that are reciprocally monophyletic must also have diagnostic characters, which would equate ESUs with species under the PSC (Table 1). Vogler and DeSalle (1994) recommended the use of diagnostic characters to define ESUs, and therefore their definition is also synonymous with species under the PSC. They submitted that the critical step in distinguishing ESUs from nonconservation units was to distinguish characters from traits. Characters (*sensu* Nixon and Wheeler 1990) are those differences that define phylogenetic lineages (fixed attributes), whereas traits (variable attributes) are those differences that indicate tokogenetic relationships. Vogler and DeSalle state that "... only characters are relevant in determining conservation units" (ESUs). Application of this criterion, while consistent with a phylogenetic framework, also fails to discriminate between ESUs (*sensu* Vogler and DeSalle) and species that are also phylogenetic lineages defined by fixed attributes.

If allowed to persist, this philosophically untenable situation will result, and most likely already has resulted in recognizing "real" species as subspecific taxa (ESUs). This would in turn result in an underestimation of biological diversity since it is species that are enumerated when biotic surveys are conducted. The conflation of species and ESUs should be of great concern to those interested in the conservation of invertebrate taxa. Protection as endangered species under the ESA is conferred only to vertebrate ESUs. For example, populations of freshwater mussels that have been identified as ESUs under any definition, would gain no protection under the ESA.

A CASE STUDY

In an investigation of the phylogenetic relationships of the freshwater mussel genus *Potamilus*, Roe and Lydeard (1997) identified two phylogenetically distinct populations of the federally threatened inflated heelsplitter (*Potamilus inflatus*) (Federal Register 1992). *Potamilus inflatus* was once distributed across a sub-

TABLE 1.
Criteria for ESU and species concepts

Evolutionarily Significant Units

Waples (1991): A population or group of populations that (1) is substantially reproductively isolated from other conspecific population units and (2) represents an important component in the evolutionary legacy of the species.

Moritz (1994): ESUs should be reciprocally monophyletic for mt DNA alleles and show significant divergence of allele frequencies at nuclear loci.

Vogler and DeSalle (1994): Populations that do not overlap in the composition of the members are diagnosably distinct, and represent separate ESUs. Attributes that confer these distinctions are diagnostic characters.

Species Concepts

Biological Species Concept

Mayr and Ashlock (1991): A species is a group of interbreeding natural populations that is reproductively isolated from other such groups.

Phylogenetic Species Concept

Cracraft (1983): The smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent.

Wheeler & Platnick (in press): The smallest aggregation of populations or lineages diagnosable by a unique combination of character states.

Recognition Species Concept

Paterson (1993): A species is that most inclusive population of individual, biparental organisms that have a common fertilization system.

Evolutionary Species Concept

Simpson (1961), Wiley (1978): An entity composed of organisms that maintains its identity from other such lineages and has its own independent evolutionary tendencies and historical fate.

stantial portion of the southeastern United States. Originally described from the Alabama River (Lea, 1831), specimens have also been collected from the Coosa, Black Warrior, and Tombigbee rivers in Alabama. In Alabama, known populations of *P. inflatus* are limited to the Black Warrior River below the Oliver Lock and Dam, although a single live specimen was recovered from the Sipsey River, a tributary of the Tombigbee River (S. McGregor, pers. comm.). In Mississippi, *P. inflatus* was last reported from the Pearl River in 1911 (Frierson 1911). Recently, "fresh dead" shells of *P. inflatus* have been recovered from the Pearl River (George et al. 1995) but to date no live animals have been found. In Louisiana, *P. inflatus* occurred in both the Amite and Tangipahoa rivers, but it is now restricted to the lower and middle portions of the Amite River. The U.S. Fish and Wildlife Service currently recognizes *P. inflatus* as a threatened species because of the reduction in the range of this species due to habitat degradation and continued threats to the remaining populations (U. S. Fish and Wildlife Service 1992).

As part of a phylogenetic analysis of the genus *Potamilus* based on ~600 base pair portion of the first subunit of the mitochondrial cytochrome oxidase c gene, Roe and Lydeard (1997) also assessed the degree of genetic differentiation in the remaining populations of *P. inflatus*. Such information was deemed useful for conservation efforts aimed at preserving the inflated heelsplitter in those rivers where it persisted. The analysis included 24 individuals. Two specimens of each species of *Potamilus* were included, with the exception of *P. inflatus* which was represented by four individuals from the Amite and Black Warrior rivers, respectively. Limited numbers of *P. inflatus* were available from the USFWS because of their threatened status. The results of the study indicated that the Amite and Black Warrior populations of *P. inflatus* represented phylogenetically and evolutionarily distinct entities. Table 2 shows that both populations of *P. inflatus* form reciprocally monophyletic groups.

Bootstrap values based on 200 replicates indicate a high degree of support for those nodes that support the Amite and Black Warrior populations as distinct (Fig. 1). In an effort to characterize the degree of differentiation observed, genetic differentiation was assessed using the number and kind of nucleotide substitutions as well as genetic distances. Examination of nucleotide substitution patterns indicates that populations of *P. inflatus* differ from each other by a total of 12 substitutions (Fig. 1). Closer inspection

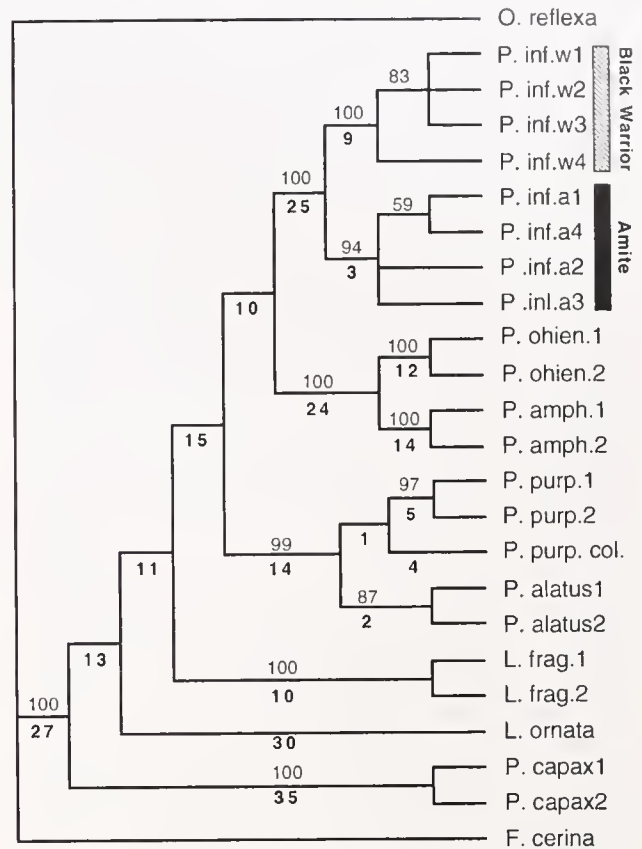


Figure 1. One of two equally parsimonious cladograms based on maximum parsimony analysis weighting transversions 2X transitions at the third codon position. Numbers above the branches correspond to the percentage of bootstrap replicates where the clade was found (200 total replicates). Only values greater than 50% are shown. Boldface numbers below the branches correspond to the number of nucleotide substitutions at those nodes. The two trees differ only in the placement of *P. p. coloradoensis* relative to *P. alatus* and *P. purpuratus*. Taxon abbreviations: *P. inf. w1-4*, *Potamilus inflatus*-Black Warrior River; *P. inf. a1-4*, *Potamilus inflatus*-Amite River; *P. purp. 1-2*, *Potamilus purpuratus*; *P. purp. col.*, *Potamilus purpuratus coloradoensis*; *P. alatus 1-2*, *Potamilus alatus*; *P. capax1-2*, *Potamilus capax*; *P. ohien. 1-2*, *Potamilus ohioensis*; *P. amph. 1-2*, *Potamilus amphichaenus*; *L. frag. 1-2*, *Leptodea fragilis*; *L. ornata*, *Lampsilis ornata*; *O. reflexa*, *Obliquaria reflexa*; *F. cerina*, *Fusconaia cerina*.

TABLE 2.

Diagnosable nucleotide substitutions for Amite and Black Warrior river populations of *P. inflatus*

Taxa	Sites											
	1	1	1	2	2	2	3	4	4	5		
	3	6	0	4	4	5	7	8	1	8	9	1
	6	6	5	4	7	8	9	2	5	3	8	3
Warrior 1	A	G	G	G	A	A	G	A	A	G	A	G
Warrior 2	?	.	.
Warrior 3
Warrior 4
Amite 1	T	A	A	A	G	G	A	G	C	A	G	C
Amite 2	T	A	A	A	G	G	A	G	C	A	G	C
Amite 3	T	A	A	A	G	G	A	G	C	A	G	C
Amite 4	T	A	A	A	G	G	A	G	C	A	?	C

? indicates missing data.

reveals that all of these substitutions represent diagnostic characters (Table 2). When substitution patterns are examined across the cladogram we observe that the number of substitutions that support the distinctness of the Amite and Black Warrior populations of *P. inflatus* are intermediate between those that distinguish *P. amphichaenus* and *P. ohioensis* and those that support *P. alatus* and *P. purpuratus* as distinct entities. Examination of genetic distances based on Kimura's "two parameter" model also reveal that the Amite and Black Warrior *P. inflatus* are more genetically distinct from each other (1.93–2.62%) than are *P. alatus* and *P. purpuratus* (1.22–1.40%), which are generally recognized as valid species.

Because of the relatively high degree of genetic differentiation observed between what were considered two populations of the same species and the presence of diagnostic characters, Roe and Lydeard (1997) recommended that both the Amite and Black Warrior forms of *P. inflatus* be recognized as separate species.

DISCUSSION

The degree of differentiation observed between the Amite and Black Warrior forms of *P. inflatus* raises the question of what these biological entities represent. Are they well differentiated populations of the same species and therefore ESUs, or are they in fact separate species? Examination of the ESU concept of Waples (Table 1) reveals that populations of *P. inflatus* meet those criteria and therefore should be considered ESUs. They are functionally reproductively isolated from each other, and represent significant components of the evolutionary legacy of the species.

Reproductive isolation of allopatric populations has presented a problem to adherents of the BSC, as pointed out by Vogler and DeSalle (1994). In most cases the distribution of observable characters is used to infer reproductive isolation. In regard to the Amite and Black Warrior populations of *P. inflatus*, potential reproductive isolation has not been assessed. The populations are functionally reproductively isolated, however, as they occur in separate river drainages. The unique reproductive cycle of *P. inflatus* and other unionacean bivalves involves the parasitization of a fish host by the bivalve larvae. It is therefore conceivable that a fish host carrying larvae could facilitate gene flow between allopatric populations of unionids. The only host identified in the case of *P. inflatus* is the freshwater drum (*Aplodinotus grunniens*) (Roe et al. 1997). Absence of records of freshwater drum from the Mobile Bay system (Metee et al. 1996) indicate a lack of salt water tolerance, and that therefore, movement of drum carrying *P. inflatus* larvae between the Black Warrior and Amite Rivers is unlikely. Reproductive isolation is further evidenced by the lack of shared mitochondrial haplotypes.

Although we have not tested for reproductive isolation in the strict sense, we have established an absence of gene flow to the degree typically required to infer the species status of allopatric populations under the BSC. The criteria of Vogler and DeSalle (1993) (Table 1) is also met in that both populations are defined by diagnosable characters and are therefore ESUs under their definition. Moritz (1994) proposed genetic criteria for determining if populations represented ESUs (Table 1). Examination of nuclear loci has not been performed on these taxa and therefore whether or not they show significant divergence for the nuclear genome remains unknown. Both populations are, however, reciprocally monophyletic for mitochondrial alleles. Based on these findings we feel a strong case can be made for the recognition of these two entities as ESUs.

Application of the criteria of the several contemporary species concepts used in this paper (Table 1) reveals that an equally strong case can be made for recognizing each population as a separate species. The Amite and Black Warrior *P. inflatus* satisfy four of the five species concepts presented here, the sole exception being the recognition species concept of Patterson (1993). The Amite and Black Warrior *P. inflatus* are reproductively isolated as required by the BSC; they are also the smallest clusters of individual organisms that are diagnosable by unique sets of character combinations and therefore satisfy the criteria of the PSC concepts included here. Additionally, they meet the criteria of the ESC: both populations have maintained their identity over time and space as evidenced by DNA sequence differences and because of their geographic isolation have their own independent evolutionary tendencies and historical fate. At the present time the presence of a "common fertilization system" has not been assessed; therefore, whether or not this criterion of the RSC is met remains unknown.

The case study presented highlights the importance of a phylogenetic perspective in identifying natural groups (i.e., species). In the absence of a phylogenetic analysis that includes other species of *Potamilus*, recognition of the Amite and Black Warrior populations as distinct evolutionary entities would be more problematic, because the assumption that they were a single species would remain untested. Recognition of these two populations as separate species does nothing to alter their levels of protection under the ESA, as *P. inflatus* is already listed as threatened by the U.S. Fish and Wildlife Service. However, as stated earlier, protection of ESUs is not extended to invertebrate populations under the ESA. Therefore, the only way to provide this level of legal protection to invertebrates in general is to recognize them as species.

The conflation of the ESU and species concepts presents a difficult problem to solve and still retain both concepts. Before we attempt to correct the problems associated with the ESU concept we should first ask, as Mayden and Wood (1995) did "... is there really a need for a 'biolegal' ESU concept when, by the nature of its conceptualization, the entities termed ESUs actually qualify as species?" If the answer to this question is no, then any conceptual problems are immediately solved. If on the other hand, the answer is yes, modification of the ESU so that it applies strictly to non-species level taxa is required. One solution would be to use criteria such as arbitrary values of genetic differentiation over which populations are recognizable as ESUs. The problem associated with such "cutoff" values is that not all lineages evolve at similar rates and a high index of genetic differentiation for one group of taxa may reflect an inconsequential degree of differentiation for another.

Perhaps the simplest solution, which allows retention of the ESU concept is to abandon the attempt to define ESUs as formal taxa and to restrict its application to those organisms for which it was originally defined (i.e., salmon stocks and other organisms with life history characteristics that make them particularly vulnerable to extinction). A modified definition of the ESU might explicitly include stocks of anadromous fishes, which spend large portions of their lives at sea where regulations regarding fishing limits are more difficult to enforce, and which have shown a consistent decline in number of individuals in consecutive years. Other characteristics might include, but are not limited to, delayed age to sexual maturity as seen in long-lived organisms such as turtles, or reliance on a rare host species for completion of their life cycle, as observed in unionid mussels.

As biodiversity continues to decline it becomes increasingly critical that we intelligently and efficiently direct resources to those problems where they are most needed and to where the most benefit can be gained. Because taxonomic/systematic classifications often determine priorities for protecting endangered species, accurate identification of biological entities is critical for the intelligent use of limited resources allocated for preserving biodiversity. Neglect of distinct taxa, whether through ignorance or poor legislation, may lead to their extinction, as is likely the case of the tuatara *Sphenodon punctatus reischekii* Wettstein in New Zealand (Daugherty et al. 1990). Because of conflation with several species concepts, application of the ESU concept, however well intended, has the potential to hinder rather than aid in the recognition of biodiversity by treating two or more distinct biological entities as a single species. The incorrect identification of biological entities can serve only to bias our efforts to protect, understand, and preserve the biological diversity of this planet which we hold in trust for future generations.

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GENETIC STRUCTURE OF *QUADRULA QUADRULA* (BIVALVIA: UNIONIDAE): LITTLE VARIATION ACROSS LARGE DISTANCES

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ABSTRACT North American freshwater bivalves of the families Unionidae and Margaritiferidae represent one of the endangered faunas of the world. Effective management of threatened and endangered species requires knowledge not only of abundances of these species but also the degree of variation within species and the geographic distribution of this intraspecific variation. We used allozyme electrophoresis to examine the genetic structure of seven *Quadrula quadrula* populations from the Ohio, Tennessee, and Tensas Rivers. We then considered the implications of our results for the development of effective bivalve conservation strategies. Descriptive measures of genetic variation within populations are quite high (2.1 ± 0.1 (se) alleles per locus; $61.4 \pm 2.6\%$ polymorphic loci; 0.24 ± 0.01 heterozygosity) relative to other unionids. Genotype frequencies met Hardy-Weinberg expectations at all polymorphic loci. Among-population variation was low and mostly confined to differences between the Tensas River population (lower Mississippi River basin) and the Ohio River basin populations. Significant differences in allele frequencies among populations were only detected at 3 of 10 loci; no differences in allele frequencies were found among Ohio River basin populations. Genetic distances, though all small, were significantly correlated with geographic distance. Estimated gene flow was high among populations, but variation among populations did tend to follow the predictions of an isolation-by-distance model of dispersal. The low levels of among-population genetic variation are remarkable given that these populations are separated by distances as great as 2,500+ river kilometers. High levels of gene flow may ensure that within-population variation remains high and that populations do not become differentiated due to genetic drift. An optimum conservation strategy for this species in the mainstem of the Ohio River would center on the protection of a number of large populations and maintenance of corridors for dispersal of host fishes. Successful protection of threatened and endangered species requires conservation of both abundance and genetic diversity of unionids. Further work is needed to characterize general patterns of genetic structure within freshwater bivalve species.

KEY WORDS: *Quadrula*, Unionidae, genetic structure, geographic patterns, within-population variation, among-population variation, conservation strategies

INTRODUCTION

Freshwater unionoid bivalves (Unionidae and Margaritiferidae) are a principal constituent of many Nearctic riverine and lake communities. Approximately 300 taxa have been described from North America, with a large number of them endemic to the central and southeastern United States (Williams et al. 1993). As the center of diversity of this fauna, the southeastern U.S. may be considered the unionoid "equivalent" of the Amazonian rain forests, with their immense diversity of insect species. Just as the rain forests have seen an enormous loss of biodiversity, North American freshwater ecosystems have suffered precipitous declines in species richness and abundance of bivalve communities, with between 55% and 72% of North American unionoid taxa listed as extinct, threatened, or of special concern (Master 1990, Williams et al. 1993). State and federal agencies have made conservation of the North American unionoid bivalve fauna a priority, and private environmental organizations have begun to publicize the plight of these organisms (Stolzenburg 1992).

The primary threats to the survival of this immensely rich fauna are human-induced sources of stress such as habitat modification

(impoundment and channelization of rivers), commercial harvest, and pollution (Bogan 1993, Neves 1993). Recently introduced exotic species, primarily the zebra mussel (*Dreissena polymorpha*), present an additional challenge to the health of native freshwater bivalves (Haag et al. 1993). Unionid populations have declined precipitously in ecosystems such as Lakes Erie and St. Clair, where zebra mussel populations have exploded (Nalepa et al. 1996).

It is now generally accepted that preservation of biodiversity requires maintenance of genetic diversity within threatened species (Schonewald-Cox et al. 1983, Soulé 1987). By definition, populations of threatened and endangered species are often small. They exhibit genetic behavior characteristic of small populations with the "loss of genetic variation resulting in the erosion of evolutionary flexibility" (Meffe 1986). These populations are susceptible to the effects of genetic drift, with its continuing erosion of genetic variation, and to extirpation (Franklin 1980). Maintenance of genetic variation within declining populations is more difficult as these populations become fragmented and gene flow becomes restricted.

Genetic differentiation of populations within a species is a function of gene flow between the populations, with greater gene flow leading to less interpopulation variation (Slatkin 1987). The minimum spatial scale at which differentiation of populations can be detected can vary from several meters (Guttman et al. 1989) to hundreds of kilometers (Berg and Garton 1994), depending on

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gene flow and dispersal ability among the populations studied. Reports of genetic variation in freshwater bivalves of the family Unionidae have concentrated on relationships among species or genera (Davis and Fuller 1981, Kat 1983a, Hoeh 1990, Stiven and Alderman 1992, Hoeh et al. 1995) or have examined patterns within species over broad geographic areas (Kat and Davis 1984, Hoeh et al. 1995, Mulvey et al. 1997). These studies have not examined the partitioning of genetic variation within and among populations. However, if government agencies and conservation organizations are to properly manage and protect threatened and endangered unionids, "normal" patterns of intrapopulation variation and gene flow between populations must be determined. These patterns can then be used to develop conservation strategies designed to preserve genetic structure of endangered species.

In this paper, we characterize the population genetic structure of *Quadrula quadrula*, a common freshwater bivalve typical of medium to large river systems of the southeastern and midwestern U.S. Specifically, we describe allozyme variation within and among seven populations from the Ohio, Tennessee, and Tensas Rivers. The observed levels of variation are used to estimate the degree of gene flow and differentiation among populations from 3 to 2,600 river kilometers apart. We place these results in a conservation biology framework, using *Q. quadrula* as a model for endangered and threatened unionids.

MATERIALS AND METHODS

Sample Collection and Electrophoresis

Bivalves were collected from beds in the Ohio, Tennessee, and Tensas Rivers in 1993 (Fig. 1). Samples were taken from four

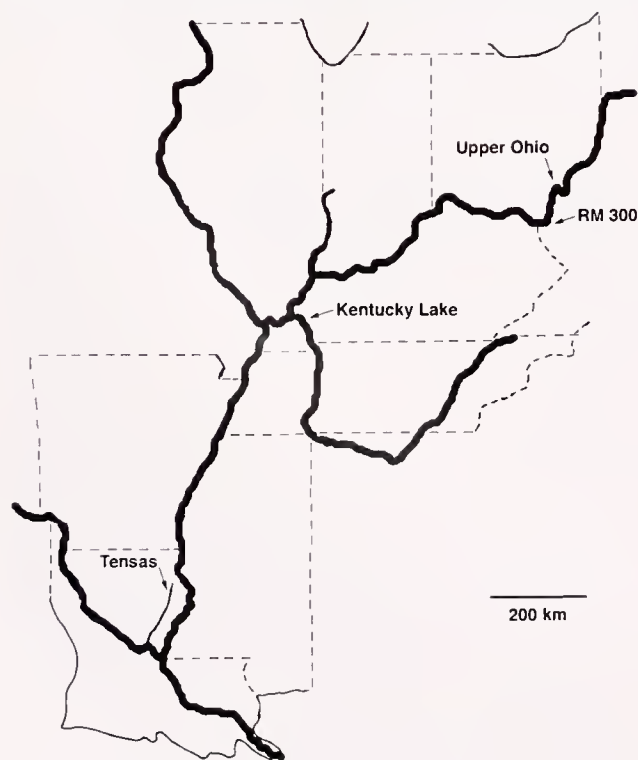


Figure 1. Populations of *Quadrula quadrula* sampled in this study. Population abbreviations follow those of the text. "Upper Ohio" sites are sites RM 238.6, RM 252.6, RM 255.3, and RM 257.7 on the Ohio River.

consecutive beds in a 31 km stretch of the Ohio River near Middleport, OH (River Mile (RM) 238.6, RM 252.6, RM 255.3, and RM 257.7) and from a site 68 km downstream near Huntington, WV (RM 300) (Fig. 1). The Tennessee River was sampled in Kentucky Lake, located 72 river km from its confluence with the Ohio River and approximately 1,100 river km from the upstream Ohio River sites. The Tensas River was sampled at Tyndel, LA, about 340 river km upstream of its confluence with the Mississippi River, more than 1,500 river km away from Kentucky Lake, and more than 2,500 river km from the Ohio River sites. Thus, within-population variation was estimated for populations across a broad geographic range within the lower Mississippi River basin, whereas among-population variation could be measured over very short distances (Ohio River) and over successively greater distances.

Individuals were collected by SCUBA, with the exception of the Tensas River, where it was shallow enough to collect while wading. At each site, 27–92 individuals were collected. For the three downstream sites, all bivalves were shucked and the mantle and adductor muscles were flash-frozen in liquid nitrogen and stored at -70°C until analyzed. For the other Ohio River populations, 20 individuals were destructively sampled, and the remainder were nondestructively sampled using a mantle biopsy (Berg et al. 1995). These tissue samples were stored as above.

Allozyme electrophoresis using starch and cellulose acetate gels was performed for eight enzyme systems, using standard recipes (Harris and Hopkinson 1976, Hebert and Beaton 1989) and buffer systems (Selander et al. 1971, Clayton and Tretiak 1972, Hebert and Beaton 1989). A total of 10 loci were resolved from these systems (Table 1). The number of loci analyzed was limited by the small amounts of tissue available for many individuals. At least 20 individuals were analyzed for all loci for each population; additional individuals were analyzed for each variable locus. Loci were considered "polymorphic" when the most common allele was present at a frequency of 0.95. Because we were often limited in the amount of tissue available from each individual and occasionally gels did not produce results, sample size varied from 3 to 92 for each locus-by-population combination (Table 2).

Statistical Analyses

Electrophoretic results were analyzed using standard population genetic techniques contained in BIOSYS-1 (Swofford and Selander 1981). Descriptive statistics calculated for each population included percent polymorphic loci, mean number of alleles per locus, and average direct-count heterozygosity. Among-population variation in mean number of alleles per locus and average direct-count heterozygosity was analyzed using one way analysis of variance. Comparison of measured genotype frequencies with Hardy-Weinberg expectations were evaluated using the "Exact Probabilities" procedure of BIOSYS-1 with the "sharper" sequential-comparison Bonferroni (s-cB) technique to adjust significance levels described by Lessios (1992). For polymorphic loci, allele frequencies among populations were compared using contingency χ^2 analysis with s-cB. Where necessary, rare alleles were pooled to meet the assumptions of this procedure.

Allele frequency differences were integrated across loci by calculating genetic distances for all pairs of populations (Nei 1978, Wright 1978). Genetic similarity of populations was determined by construction of dendrograms using modified Rogers' genetic dis-

TABLE 1.
Enzyme systems, gels, and buffers used with *Quadrula quadrula*.

Enzyme System	Number of Loci	E. C. Number	Gel Type	Buffer System	
Esterase	2	3.1.1.1	Starch	TC 6.7	1 visible locus ^a 1 UV locus ^b
Glucosephosphate isomerase	1	5.3.1.9	Starch	CT	
Malate dehydrogenase	2	1.1.1.37	Starch	CT	
Mannose phosphate isomerase	1	5.3.1.8	Starch	LiOH	
Peptidase	1	3.4.11	Cellulose	TG	leucyl-tyrosine used as dipeptide
Phosphoglucumutase	2	2.7.5.1	Starch	TC 6.7	
Superoxide dismutase	1	1.15.1.1	Starch	LiOH	

Number of loci refers to the number of scoreable allozyme loci for a system.

^a α -Naphthyl acetate used as substrate.

^b 4-Methylumbelliferyl acetate used as substrate.

CT from Clayton and Tretiak (1972); TC 6.7 and LiOH from Selander et al. (1971); TG from Hebert and Beaton (1989).

tance and the unweighted-pair-group method (UPGMA) to cluster populations (Sokal and Sneath 1963). The dendrogram used a population of *Quadrula apiculata* (Cantonwine and Berg unpubl) as an outgroup and bootstrap values for the nodes of the dendrogram were obtained from 1,000 replicates using the software package Tools for Population Genetic Analysis (TFPGA) (Miller 1998). Correlation of genetic and geographic distance was determined using a Mantel test in TFGPA, with geographic distance measured as river distances (rather than straight-line distances) between sites. Among-population genetic variation was further analyzed by calculating values of F_{ST} (θ of Weir and Cockerham 1984) for polymorphic loci. Values were bootstrapped (1,000 replicates) to obtain 95% confidence intervals. The number of migrants per generation (N_m) among populations was estimated from F_{ST} , assuming a stepping-stone model of dispersal (Slatkin and Barton 1989).

RESULTS

Within-population Variation

Seven of the 10 loci sampled were polymorphic in at least one population (Table 2). One other locus (EST-UV) was variable but not polymorphic according to the 95% criterion (see above), whereas 2 loci (MDH-1 and SOD) were fixed for a single allele in all populations (minimum of 27 individuals sampled per population). Within-population genetic variation was similar for all populations, with the percentage of polymorphic loci varying from 50% to 70% (Table 2). Mean number of alleles per locus was not significantly different among populations ($n = 10$, $F_{6,63} = 1.43$, $p = .22$), nor was average direct-count heterozygosity ($n = 10$, $F_{6,63} = 0.13$, $p = .99$). All 45 polymorphic locus-by-population combinations had genotype frequencies that were not significantly different from Hardy-Weinberg expectation at an experiment-wise error rate of $\alpha = 0.05$.

Among-population Variation

Allele frequencies did not vary significantly among all populations for four polymorphic loci (EST, GPI, MDH-2, PEP; $\chi^2 = 25.2$, $n = 12$, experimentwise error rate of $\alpha = 0.05$; Table 2). Allele frequencies varied significantly among populations for the other three polymorphic loci (MPI, PGM-1, PGM-2; $\chi^2 = 49.2$, $n = 12$, experimentwise $\alpha = 0.05$). However, when the Tensas

River population was omitted from the analysis, there were no significant differences in allele frequencies for these three loci among the remaining populations ($\chi^2 = 29.6$, $n = 10$, experimentwise $\alpha = 0.05$). For PGM-1, this was primarily due to the relatively high frequency of allele 1 in the Tensas River population and the presence of allele 2 in only the Kentucky Lake and Tensas River populations (Table 2). Allele 4 was present at relatively high frequency in the RM 300 population and at low frequency in the Tensas River population. For PGM-2, allele 2 was at least twice as common as allele 1 in all populations except the Tensas (Table 2). In the latter population, alleles 1 and 2 were present in similar frequencies. For MPI, the differences are apparently the result of the cumulative effect of differences in frequencies for each of the four alleles and the presence of allele 4 only in the two most downstream populations (Table 2). Thus, populations from the Ohio River basin do not have significant differences in allele frequencies at any polymorphic loci. For three of seven polymorphic loci, the Tensas River population has significantly different allele frequencies than the Ohio River basin populations.

Genetic distances were lowest among populations from the Ohio River basin, whereas distances between each of these populations and the Tensas River population were higher (Table 3). Cluster analysis shows that populations from the upper Ohio River are most similar; of these, the three populations that were closest geographically (RM 252.6, 255.3, and 257.7—a total of 8.3 km apart) had the lowest average genetic distances. The RM 238.6 population clustered with downstream populations (RM 300 and Kentucky Lake). These populations are more similar to other Ohio River populations than to the Tensas River population (Fig. 2). The relatively high (55.5%) bootstrap value for the Ohio Basin node reinforces the conclusion that the Tensas River population is different from these populations, whereas the generally low bootstrap values at the other nodes within the Ohio Basin are consistent with a lack of differentiation among these populations. The tight positive correlation of genetic distance with geographic distance ($r = 0.83$, $n = 21$, $p = .003$; Fig. 3) matches the predictions of an isolation-by-distance model of dispersal. Values of F_{ST} were low but significantly greater than zero over all populations; exclusion of the Tensas River population resulted in even lower, but still significant, values (Table 4). The mean value of N_m was 2.4 times larger when the Tensas River population was excluded from the analysis.

TABLE 2.

Allele frequencies at variable loci and descriptive measures of within-population genetic variation for *Quadrula quadrula*.

Locus	Allele	Population					Kentucky Lake	Tensas River
		RM 238.6	RM 252.2	RM 255.3	RM 257.7	RM 300		
EST ^a	1	—	—	—	—	—	0.01	—
	2	1.00	1.00	1.00	1.00	0.98	0.93	0.94
	3	—	—	—	—	0.03	0.06	0.06
	(n)	27	48	46	47	40	75	92
EST-UV	1	—	—	—	—	—	0.01	—
	2	0.98	1.00	1.00	1.00	1.00	0.97	0.98
	3	0.02	—	—	—	—	0.01	0.02
	4	—	—	—	—	—	0.01	—
	(n)	27	43	47	47	40	74	88
GPI ^a	1	—	—	—	—	—	—	0.02
	2	0.37	0.26	0.32	0.31	0.30	0.20	0.21
	3	0.63	0.74	0.68	0.69	0.71	0.80	0.77
	(n)	27	46	47	50	39	74	90
MDH-2 ^a	1	0.24	0.27	0.36	0.26	0.26	0.36	0.26
	2	0.76	0.73	0.64	0.75	0.74	0.64	0.74
	(n)	27	45	47	49	36	73	88
	3	0.16	0.34	0.33	0.34	0.15	0.31	0.12
MPI ^{a,b}	2	0.54	0.31	0.29	0.39	0.51	0.47	0.51
	3	0.30	0.35	0.38	0.27	0.34	0.21	0.36
	4	—	—	—	—	—	0.01	0.01
	(n)	25	40	41	41	40	75	73
PEP ^a	1	—	—	—	—	—	—	0.01
	2	0.50	0.36	0.41	0.50	0.59	0.60	0.64
	3	—	—	—	—	0.03	—	0.01
	4	0.50	0.64	0.59	0.50	0.39	0.40	0.33
	(n)	3	14	16	13	40	57	39
PGM-1 ^{a,b}	1	0.03	0.03	0.03	0.03	0.08	0.07	0.29
	2	—	—	—	—	—	0.01	0.02
	3	0.91	0.91	0.94	0.97	0.75	0.87	0.68
	4	0.06	0.05	0.03	—	0.17	0.05	0.02
	(n)	16	29	36	35	36	59	64
PGM-2 ^{a,b}	1	0.22	0.23	0.22	0.26	0.29	0.33	0.52
	2	0.78	0.77	0.78	0.73	0.71	0.67	0.47
	3	—	—	—	0.01	—	—	0.01
	(n)	27	46	46	48	40	75	92
Mean sample size per locus		23.3 (2.5)	39.7 (3.3)	40.8 (3.1)	41.3 (3.6)	66.5 (4.8)	37.2 (1.9)	74.4 (7.7)
Mean number of alleles per locus		1.9 (0.2)	1.8 (0.2)	1.8 (0.2)	1.8 (0.2)	2.5 (0.4)	2.0 (0.3)	2.6 (0.4)
Polymorphic loci (%)		60	60	60	50	70	60	70
Mean heterozygosity		0.26 (0.09)	0.22 (0.07)	0.24 (0.08)	0.20 (0.07)	0.27 (0.07)	0.23 (0.07)	0.27 (0.07)

Two additional loci (MDH-1 and SOD) were fixed for the same allele in all populations. Population abbreviations follow those of the text. Numbers in parentheses are standard errors. (n) = sample size.

^a Polymorphic locus (95% criterion).

^b Significant variation in allele frequencies (contingency chi-square, $p < 0.05$) when Tensas River population is included.

DISCUSSION

Within-population Variation

Our study shows that populations of *Quadrula quadrula* may contain significant amounts of within-population genetic variation. A study of *Q. quadrula* in Arkansas rivers found considerably lower levels of within-population variation and higher levels of differentiation among populations (Johnson et al. 1998). The grand mean of direct-count heterozygosity for *Q. quadrula* from our study was 0.24 (range 0.20–0.27), whereas Arkansas populations showed an average heterozygosity of 0.06 (range 0.04–0.07). In fact, we report some of the highest levels of genetic variation found in unionid populations when compared with other published studies (Badino 1982, Davis 1984, Davis and Mulvey 1993, Kat

1983b, Stiven and Alderman 1992, van der Bank 1995, Nagel et al. 1996, Hoeh et al. 1998, Johnson et al. 1998). The grand mean of average heterozygosity from these other studies of unionids is only 0.09 (range 0.00–0.31 for 44 studies). Almost all (91%) previously reported values of heterozygosity were lower than the minimum value for the *Q. quadrula* populations we sampled. A similar situation occurs when comparing average number of alleles per locus and proportion of polymorphic loci among populations. Based on these comparisons, the populations of *Q. quadrula* that we sampled contain high levels of genetic variation compared with other unionid populations. Our values are similar to those reported for the giant marine clams *Tridacna gigas* and *T. maxima* (Benzie and Williams 1992a, Benzie and Williams 1992b). Reports across large numbers of taxa show that *Q. quadrula* contains above-

TABLE 3.
Matrix of genetic distances among pairs of populations of *Quadrula quadrula*.

Population	Ohio RM 238.6	Ohio RM 252.2	Ohio RM 255.3	Ohio RM 257.7	Ohio RM 300	Kentucky Lake	Tensas River
Ohio RM 238.6	—	0.000	0.000	0.000	0.001	0.000	0.017
Ohio RM 252.2	0.087	—	0.000	0.000	0.010	0.011	0.035
Ohio RM 255.3	0.087	0.040	—	0.000	0.010	0.012	0.037
Ohio RM 257.7	0.062	0.058	0.059	—	0.003	0.005	0.025
Ohio RM 300	0.065	0.111	0.112	0.089	—	0.004	0.015
Kentucky Lake	0.094	0.104	0.101	0.073	0.077	—	0.010
Tensas River	0.143	0.170	0.173	0.147	0.112	0.101	—

Nei's (1978) unbiased genetic distances are above the diagonal and modified Rogers' distances (Wright 1978) are below the diagonal. Distances calculated using 10 allozyme loci. Population abbreviations follow those of the text.

average levels of variation within populations (Nevo 1978). The high degree of within-population genetic variation implies that these populations of *Q. quadrula* have large effective population sizes and that there have not been significant genetic bottlenecks in the recent past.

Genotype frequencies of *Quadrula quadrula* populations were not significantly different from Hardy-Weinberg expectation (HWE). We have found similar results for *Elliptio dilatata* from streams in Ohio (Berg and Guttman, unpubl). Other studies of unionids have shown considerable variation in the proportion of loci that show significant differences from HWE. Among three species of unionids in North Carolina, the proportion of loci deviating from HWE varied between 5% and 75%, and all of these deviations were heterozygote deficiencies (Stiven and Alderman 1992). Genotype frequencies of Arkansas unionids, including *Q. quadrula* and *Q. pustulosa*, were different from HWE in 67% of polymorphic locus-by-population combinations (Johnson et al. 1998), with most of these deviations being heterozygote deficits. A similar pattern was seen in European anodontines, where 64% of polymorphic locus-by-population combinations showed heterozygote deficits (Nagel et al. 1996). In the genus *Utterbackia*, the gonochoric species *U. peggyae* and *U. peninsularis* exhibited deviation from HWE at only 6% and 8% of all locus-by-population combinations, respectively (Hoeh et al. 1998). In contrast, two simultaneously hermaphroditic species, *U. imbecillis* and an un-

described *U. "imbecillis,"* showed heterozygote deficits at 65% and 50% of all locus-by-population combinations. Hoeh et al. (1998) concluded that deviations from HWE, in particular heterozygote deficiencies, may be explained by the mating systems employed by unionid species (e.g., self-fertilization versus cross-fertilization). Thus, our results are completely consistent with those expected of a gonochoric species with high levels of gene flow among populations.

Among-population Variation

Variation among populations was quite low: the vast majority of this among-population variation occurs between the Tensas River site and the sites in the Ohio River basin. Even over river distances of >1000 km, no differences in allele frequencies were found among populations in the Ohio River basin, whereas the Tensas River site differed from the Ohio River populations at only three of seven polymorphic loci. Gene diversity analyses show that little of the total heterozygosity within *Quadrula quadrula* is accounted for by among-population variation. When compared with other studies of unionids, the differences between the Tensas River population and the Ohio basin populations of *Q. quadrula* were low. Genetic distances among populations in this study averaged 0.009 (range <0.0005–0.037) for all populations; exclusion of the Tensas River site lowered average distance to 0.004 (<0.0005–

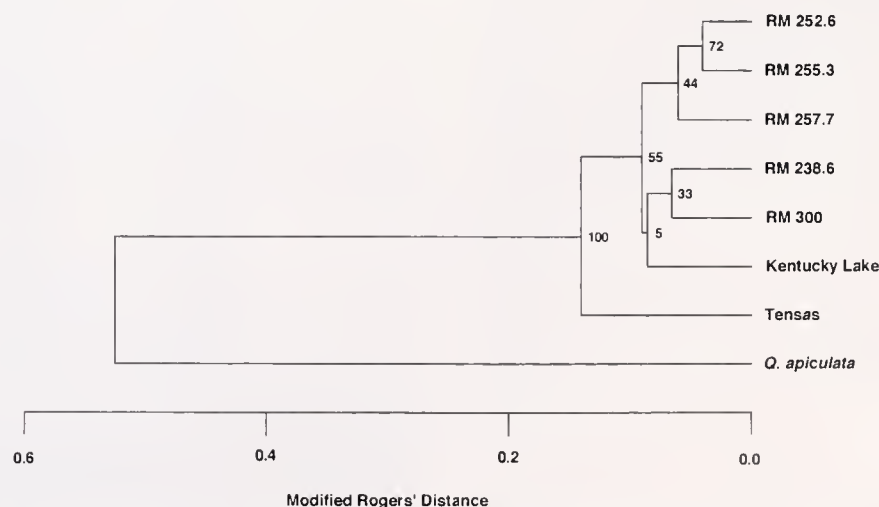


Figure 2. Modified Rogers' genetic distances (Wright 1978) for populations of *Quadrula quadrula*. Distances calculated using 10 allozyme loci. Values at the nodes represent the percentages of 1,000 bootstrapped trees that produced these nodes.

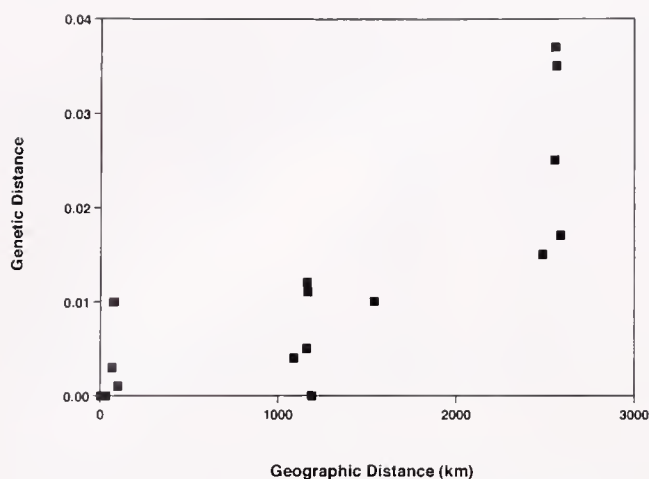


Figure 3. Correlation of unbiased genetic distance (Nei 1978) and geographic distance (river km) between pairs of populations of *Quadrula quadrula*. The correlation is significant (Mantel test; $r = 0.83$, $n = 21$, $p = .003$).

0.012). Published measures of unbiased genetic distance (Nei 1978) among unionid populations averaged 0.047 (0.000–0.252) for six species (Nagel et al. 1996, Stiven and Alderman 1992). Three populations of *Anodonta cygnea* showed little differentiation (mean genetic distance of 0.008; range 0.000–0.012) over large geographic distances (Nagel et al. 1996). However, other species of *Anodonta* reported from the same study showed mean values between 0.063 and 0.108 among populations within the same river basin. Most published studies of unionid genetic structure have calculated standard genetic distance (Nei 1972) rather than unbiased genetic distance. Comparison of our calculations of standard genetic distance with those of such studies (Davis and Mulvey 1993, Davis et al. 1981, Kat 1983a, Badino 1982) show that genetic distances among populations of *Q. quadrula* are very low, even though populations were separated by as much as 2,500+ river kilometers.

The dendrogram created from the cluster analysis and the tight correlation of genetic distance with geographic distance are both consistent with isolation-by-distance (Wright 1943, Slatkin 1993); this process increases genetic differentiation as populations become separated by larger distances. Given the method of dispersal available to unionids (downstream transport of spermatozeugmata, host fish transport of glochidia larvae up and down rivers), this isolation-by-distance likely arises due to a one-dimensional stepping-stone pattern of dispersal (Slatkin 1985). However, the degree of gene flow among populations is apparently sufficient (greater than 1 migrant per generation) to prevent significant differentiation of populations by genetic drift (Slatkin and Barton 1989). Gene flow estimates among populations of *Anodonta anatina* from several European Atlantic coast drainages were sufficient for the authors of the study to classify this species as “panmictic” (Nagel et al. 1996), although the values reported were much lower than we report (*A. anatina* mean $N_m = 1.2$, *Quadrula quadrula* mean $N_m = 7.8$).

Genetic Structure of *Quadrula quadrula*

Our results suggest that populations of *Quadrula quadrula* have high levels of gene flow within the Ohio and lower Missis-

sippi River basins and that this has two consequences for genetic structure of these populations. The first is that this gene flow maintains high levels of within-population genetic variation relative to that seen in other unionids, and genetic drift does not play a significant role in determining the frequencies of alleles within populations. Secondly, the high levels of gene flow inhibit differentiation among populations. What little differentiation that has occurred is a function of isolation-by-distance. Because gene flow among unionid populations over spatial scales greater than a few meters is a function of host fish movements, our results suggest that there is sufficient movement of host fishes to allow glochidia to disperse to at least the next bed in a river. The flathead catfish (*Pylodictis olivaris*) is a known host of *Q. quadrula*, and other large-river species within the genus *Quadrula* utilize various catfishes and large centrarchids (crappie—*Pomoxis* spp.; largemouth bass—*Micropterus salmoides*) (Watters 1994). Because these hosts are all common large-river species, they are capable of movement among beds that are separated by distances greater than a kilometer. Such minimal dispersal would then be best described by a one-dimensional stepping-stone model. It is certainly possible that such host fishes may move sufficient distances to “skip” adjacent beds and thus disperse their glochidia much greater distances. As a result, we can best characterize the genetic structure of *Q. quadrula* in the Ohio and lower Mississippi basins as a single “metapopulation” (Hastings and Harrison 1994) in which gene flow prevents “extinction” of some alleles and fixation of others.

Genetic structure is known to be a function of dispersal ability in other molluscs. Highly vagile species such as the zebra mussel (*Dreissena polymorpha*) show low values of F_{ST} (Marsden et al. 1995). Giant clams (*Tridacna gigas* and *T. maxima*) show high levels of within-population variation, few deviations of allele frequencies from Hardy-Weinberg expectations, and little or no ge-

TABLE 4.

F_{ST} (Θ of Weir and Cockerham 1984) values for polymorphic loci and average migration rates.

Populations	Locus	Fst	Nm
All	EST	0.024	
	GPI	0.010	
	MDH-2	0.003	
	MPI	0.026	
	PEP	0.018	
	PGM-1	0.089	
	PGM-2	0.064	
	Overall	0.031 (0.013–0.056)	7.815
Ohio R. Basin	EST	0.034	
	GPI	0.010	
	MDH-2	0.004	
	MPI	0.019	
	PEP	0.032	
	PGM-1	0.042	
	PGM-2	0.009	
	Overall	0.013 (0.006–0.020)	18.981

N_m —number of migrants per generation for populations of *Quadrula quadrula*. Comparisons are among all populations and among populations from the Ohio River basin (Tensas River population excluded). Numbers in parentheses are 95% confidence intervals from bootstrapping of 1,000 replicates.

netic differentiation among populations in the south Pacific Ocean (Benzie and Williams 1992a, Benzie and Williams 1992b). Where genetic differentiation occurs, the patterns are consistent with isolation-by-distance. Marine snails of the genus *Littorina* exhibit a strong negative correlation between genetic differentiation among populations and dispersal ability (Janson 1987). The results of our study show that although life histories of marine molluscs and freshwater unionoid bivalves are quite different, *Quadrula quadrula* has genetic structure similar to that of zebra mussels, giant clams, and littorinids with planktonic larvae. In all of these cases, high variation within populations and little differentiation among populations are likely due to high levels of gene flow between populations.

Conservation Implications

As the field of conservation biology has grown, efforts have been made to consider genetic variation when developing strategies for management and conservation of rare species (reviewed in Avise 1994). Recent work has focused on providing a conceptual framework of the organization of genetic variation in species such that management agencies and conservation organizations are able to design strategies that conserve genetic diversity. A key piece of this conceptual framework is the definition of Evolutionarily Significant Units (ESUs) and Management Units (MUs) within ESUs (Moritz 1994). ESUs tend to be concordant with morphological species, and MUs are subdivisions within ESUs. With this approach, MUs are defined "... as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles" (Moritz 1994). The results of our study show that *Quadrula quadrula* in the Ohio and lower Mississippi basins is composed of at least 2 MUs—one in the mainstem of the Ohio and lower Tennessee Rivers and one in the Tensas River. However, it is possible that additional MUs were undetected because of the great distance between Kentucky Lake and the Tensas River, and because we did not sample the mitochondrial genome. Conservation of genetic variation within ESUs implies the protection of each MU within it. Thus, a conservation strategy designed to protect *Q. quadrula* or any other unionid species with similar genetic structure would need to "customize" efforts for each MU.

Understanding the genetic structure of *Quadrula quadrula* in the Ohio and lower Mississippi basins provides insight for the development of effective conservation strategies for this species and those threatened and endangered (T&E) species that have similar genetic structure. Our results suggest that any single population of *Q. quadrula* contains most of the genetic diversity of the populations along the mainstems of the Ohio and lower Tennessee Rivers. As such, only a small number of populations scattered

throughout this stretch of rivers would need to be conserved to ensure conservation of most of the total genetic diversity within this region. However, each of these populations must be large enough that they are able to maintain the high levels of within-population variation. Under these circumstances, an optimal conservation strategy would be to protect a number of populations, each of which remains large. Such a strategy would be compatible with the establishment of sanctuaries at multiple locations along a river system and the maintenance of corridors for movement of host fishes among these sanctuaries. The distances between these sanctuaries should then be a function of host fish dispersal distances. This type of strategy would also work for T&E species that are locally abundant but limited to only a small number of populations. Of course, populations of T&E species are often quite small and in these cases, efforts must be made to protect all individuals.

Recent studies have noted declines in many species of unionids, not just those that are listed as threatened or endangered (Neves 1997, Howells et al. 1997, other studies reported in Cummings et al. 1997). If government agencies and other conservation organizations are to be successful in protecting species so that listing is not necessary, steps must be taken to conserve both abundance and genetic diversity of unionids. This effort requires an understanding of the genetic structure of these species and thus, there is a great need for additional studies to determine whether the model of genetic structure we present is generally applicable to unionids or whether other patterns exist. If other patterns are found, the implications for development of conservation strategies for species exhibiting these patterns must be explored. There is no doubt that understanding the genetic structure of unionid species is essential if this unique part of North America's freshwater fauna is to be preserved.

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GENETIC DIVERSITY AND CELLULOLYTIC ACTIVITY AMONG SEVERAL SPECIES OF UNIONID BIVALVES IN ARKANSAS

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ABSTRACT Allozyme analysis was utilized to determine the genetic diversity of four species of bivalves in the Subfamily Ambleminae (*Amblema plicata*, *Plectomerus dombeyanus*, *Quadrula pustulosa*, and *Q. quadrula*) in the Cache and White rivers of Arkansas. Bivalve populations of both rivers have been subjected to frequent harvest, whereas White River populations have been exposed to periodic habitat destruction due to dredging. Nine enzyme systems representing 16 loci were selected for analysis based upon their expression in adductor muscle. Ranges of polymorphism (P) were from 0.572 for *A. plicata* to 0.360 for *Q. quadrula*; heterozygosity (H) values ranged from 0.049 for *P. dombeyanus* to 0.144 for *Q. pustulosa*. With the exception of low genetic diversity (P, H, and number of alleles per locus) for *Q. quadrula*, genetic diversity values were similar to previous studies involving Ambleminae of other water systems. Populations were characterized by heterozygote deficiencies at all loci. Several determinants of heterozygote deficiency were investigated, with selection posed as a viable hypothesis. Cellulolytic activity from bivalves in both the Cache and White rivers was significantly reduced ($\alpha 0.05$) to as low as 12% and 28%, respectively, of upstream enzyme activities. This downstream digestive enzyme difference was most apparent in *P. dombeyanus*, whereas site reductions were not as apparent for other species sampled. Evidence of genetic decline associated with bottlenecks was identified for *Quadrula quadrula*, and this loss of genetic diversity is detrimental to the stability of bivalve populations.

INTRODUCTION

As a result of the combined anthropogenic effects of overharvesting, pollution, and destruction of habitat by impoundments, dredging, and bridge construction, the size and number of bivalve beds have steadily decreased nationwide (Williams et al. 1993). Approximately one-third of the 300 bivalve species are classified as endangered or possibly extinct (Williams et al. 1993). Researchers have reported dramatic, long-term decreases in bivalve species diversity in various river systems for several states (Matteson and Dexter 1966; Isom and Yokley 1968; Starrett 1971; Suloway 1981). Commercially harvested bivalve beds associated with Arkansas rivers have recently been described (Rust 1993; Christian 1995; Davidson 1997; Posey 1997). Christian, in a comprehensive survey during 1995, identified a 54% loss of historically documented beds in the White River of Arkansas. Decline in abundance of Arkansas bivalves has also been evidenced by a 75% reduction in harvest figures from these rivers in the 1990s alone (Todd 1994). Although reduction in overseas demand for shells has influenced the harvest, catch per unit effort by shellers has continued to decline (Todd 1994). Shellers have also indicated dramatic declines in bivalve bed sizes (K. C. Ward, E. Kohal, J. T. Easter, pers. comm.). Thus, these sediment-bound populations are responding not only to dynamic physical attributes associated with habitat (fluvial dynamics), but also to changing selective pressures for age and size specific removal (e.g., shelltakers removing larger individuals versus hydrologic forces removing smaller individuals).

Reductions in genetic diversity of populations have been associated with population bottlenecks of species (Nei et al. 1975; Avise et al. 1988; Bernatchez et al. 1989). There is evidence that genetic variability provides species with the ability to survive and adapt to changing environmental conditions (e.g., hypolimnetic release of impoundment waters in the White River) (Hedrick et al. 1976; Gillespie and Guttman 1989). In addition, a decline in locus heterozygosity associated with the glycolytic pathway and protein catabolism has been correlated with reductions in energetic efficiency and growth rates in bivalves (Koehn et al. 1976; Koehn and

Shumway 1982; Garton et al. 1984; Singh and Green 1984; Koehn et al. 1988).

The management of freshwater bivalves is a unique situation since it relies not only on an understanding of the genetic structure of these species but also the host-parasitic interactions of the glochidial stage. The effects of host fish migration during the glochidial stages greatly impacts gene flow. The additional consideration of facultative hermaphroditism in some bivalve species provides another significant life history characteristic that complicates understanding of genetic diversity.

Purpose of the Study

We compared cellulase activity and population genetic structure for four species within the Subfamily Ambleminae common to Arkansas waters. These species were *Amblema plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa* (Lea), and *Q. quadrula* (Rafinesque). All four species are widely distributed within the Mississippi River drainage and are classified as currently stable species (Williams et al. 1993). Each is commercially important with the exception of *P. dombeyanus*.

Cellulolytic activity was used to measure individual bivalve condition from sampling sites, and current patterns of genetic variability among populations were used to indicate historical exposures to a stress. Cellulase has been noted as an important enzyme of intermediary metabolism for detritivores such as unionids, and activity has been negatively correlated with chemical and biological sources of stress in freshwater bivalves (Farris et al. 1994; Haag et al. 1993). Typically, the variability observed in field-collected organisms prevents reliable measurement of the sensitivity of enzyme alteration to toxicant or physically related stress (Mayer et al. 1992). However, changes in cellulase activity in molluscs demonstrate measurable biochemical responses to pollutants and/or other stressors (Beeby 1993; Milam 1996; Farris et al. 1988).

The Cache and White rivers belong to the Mississippi River drainage system and represent significant sources of bivalves to

Arkansas shell takers. Maintenance dredging is performed annually in the White River to accommodate barge traffic, whereas only the upper reaches of the Cache River are channelized for flood control purposes (Tillman, U.S. Army Corps of Engineers, pers. comm.). Gravel removal occurs for commercial purposes in the White River.

There is an added urgency to gather information regarding the population structure and systematics for unionid species since the decline in numbers of Arkansas bivalves has also possibly reduced the genetic diversity of existing populations. No genetic studies have been performed on freshwater bivalves from Arkansas river drainages. The total absence of designated minimum viable population sizes for bivalves suggests that conservation geneticists can only extrapolate such information from species possessing more simplistic life histories. A minimal threshold population of individuals is not presently utilized by fisheries managers and the application of minimum shell size to commercial shellers is the only means for maintaining current populations.

It was our concern that the decline in numbers of Arkansas bivalves has also greatly reduced the genetic diversity of existing populations. In addition to identifying the genetic diversity of four bivalve species in Arkansas, our primary question was to determine if bivalve populations subjected to frequent harvest and habitat destruction have low genetic diversity relative to previously studied bivalve species. Our third goal was to investigate if differential heterozygosities for intraspecific populations were related to sampling or other environmental variables. Fourth, we wanted to study the cellulolytic activity of differing populations and species, and to investigate the relationship of cellulolytic activity with genetic diversity. The genetics of a population typically reflects historic responses of a population to the environment and random genetic events. The cellulolytic activity of a population reflects the ambient physiological condition of individuals which is due to both the genetics and immediate efficiency to assimilate available carbon.

METHODS

Genetic diversity and cellulolytic activity were determined within six populations for the lower regions of the Cache and White rivers. Three sequential downstream bivalve beds were chosen for each river: miles 37, 36, and 35 (sites A, B, and C, respectively) for the Cache River, and miles 63.5, 57.2, and 48.5 for the White River (sites D, E, and F, respectively). The Cache River basin drains southward along the western extent of the Mississippi Embayment and flows into the White River near Clarendon, Arkansas. Whereas the White River flows through the Ozark Highlands, the study region is best characterized by the alluvial soil deposits of the Mississippi Delta. Both streams are characterized within the study region as having low stream gradient and high turbidity and dissolved solids (Fig. 1).

Four species of bivalves were studied, with 12–36 individuals of two species collected from each site (Table 1). Hookah rig diving was used to obtain the bivalves in August and September of 1994. Specimens were brought back to the laboratory on ice and processed immediately or frozen at -70°C . Voucher specimens have been deposited in the Unionacea collection of the Arkansas State University Museum of Zoology (ASUMZ).

Genetic Analysis

Adductor muscles were homogenized in equal volumes (w/v) of Tris-HCl buffer (pH 7.0). Electrophoresis of homogenate was

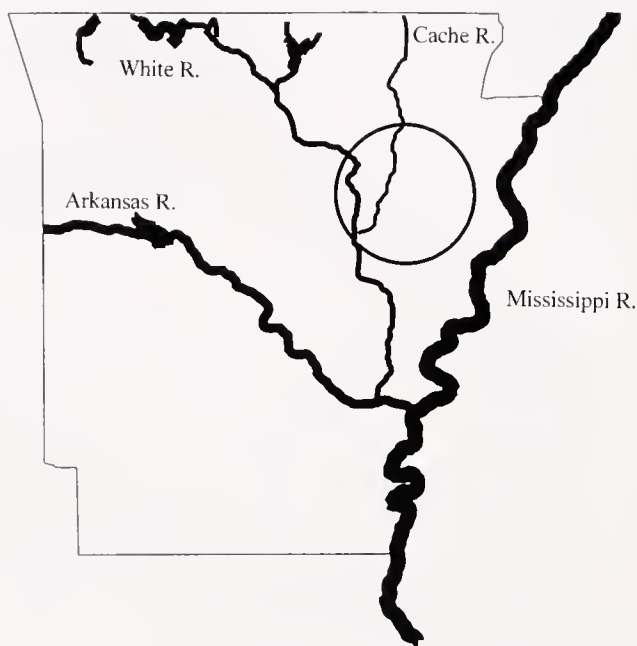


Figure 1. Study sites of the Cache (river miles 35, 36, 37) and White (river miles 48, 57, 64) rivers in Arkansas.

performed on cellulose acetate plates in TG buffer (0.025 M Tris; 0.192 M glycine). Nine enzyme systems representing 16 loci were selected for analysis based upon their expression in adductor muscle. The loci were as follows: fumarase (FUM-1, FUM-2; E.C. No. 4.2.1.2); glutamate-oxaloacetate transferase (GOT-1, GOT-2; E.C. No. 2.6.1.1); isocitrate dehydrogenase (IDH-1, IDH-2; E.C. No. 1.1.1.42); lactate dehydrogenase (LDH-1, LDH-2; E.C. No. 1.1.1.27); malate dehydrogenase (MDH-1, MDH-2; E.C. No. 1.1.1.37); malic enzyme (ME-1, ME-2; E.C. No. 1.1.1.40); man- nose phosphate isomerase (MPI-1, MPI-2; E.C. No. 5.3.1.8); phosphoglucose isomerase (PGI-1; E.C. No. 5.3.1.9); phosphoglucomutase (PGM-1, PGM-2; E.C. No. 2.7.5.1) (IUBNC 1984). The distance of migration for each specific enzyme was visualized by histochemical staining (Hebert and Beaton 1989).

Cellulolytic Enzyme Assay

Individuals were thawed and subsamples (visceral mass) of whole-body dissections were used in the analysis for cellulolytic enzyme activity (Farris et al. 1989). The samples were homogenized in phosphate buffer at pH 6.1 at a wet mass to buffer ratio of 0.02 g/ml. Samples were centrifuged for 15 min at 15,000 rpm, supernatants were decanted for endo/exocellulase estimates (and their synergistic interaction), and the pellets were recovered for dry mass measurements. Cellulase activity was expressed as a relativized product index to compare units of activity for bivalve species and river locations. One unit of the enzyme is defined in this context as the amount of enzyme required to liberate 1 mg of reducing sugar equivalent to that of glucose per h using carboxymethylcellulose (CMC) as a substrate.

Statistical Analysis

Mean sample size per locus, mean number of alleles per locus, polymorphism, mean heterozygosity, Hardy-Weinberg (H-W) equilibrium (D statistics), contingency chi-square analysis, and Wright's statistics (F_{IS} , F_{IT} , F_{ST}) were determined using the pro-

TABLE 1.

Genetic variability of *A. plicata*, *P. dombeyanus*, *Q. pustulosa* and *Q. quadrula* in three sites each in the Cache (CR-A, B, and C) and White (WR-D, E, F) rivers, Arkansas.

Species	Collecting Sites	Sample Size	Heterozygosity	Polymorphism	Alleles/Locus
<i>A. plicata</i>	CR-A	35	0.167 (0.081)	0.857	2.6 (0.3)
	CR-B	24	0.064 (0.044)	0.286	1.7 (0.3)
	Population average	30	0.116 (0.052)	0.572 (0.286)	2.1 (0.3)
<i>P. dombeyanus</i>	CR-A	31	0.042 (0.024)	0.769	2.5 (0.2)
	CR-B	18	0.056 (0.023)	0.538	1.8 (0.2)
	CR-C	36	0.049 (0.049)	0.077	1.1 (0.1)
<i>Q. pustulosa</i>	Population average	28	0.049 (0.006)	0.461 (0.288)	1.8 (0.2)
	WR-D	12	0.188 (0.092)	0.375	1.4 (0.1)
	WR-E	20	0.128 (0.057)	0.500	1.8 (0.2)
<i>Q. quadrula</i>	WR-F	15	0.117 (0.057)	0.438	1.8 (0.3)
	Population average	16	0.144 (0.031)	0.438 (0.051)	1.6 (0.2)
	WR-D	32	0.062 (0.033)	0.375	1.6 (0.2)
	WR-E	24	0.069 (0.028)	0.438	1.9 (0.3)
	WR-F	36	0.060 (0.031)	0.375	1.7 (0.3)
	CR-C	36	0.041 (0.027)	0.250	1.4 (0.2)
	Population average	32	0.058 (0.010)	0.360 (0.068)	1.6 (0.2)

Standard errors are in parentheses.

gram BIOSYS-1 (Swofford and Selander 1989). Levene's (1949) correction for small sample size was utilized as appropriate in the determination of H-W equilibrium. The sequential-comparison Bonferroni technique was used to adjust significance levels for determination of H-W equilibrium to reduce Type I error (Lessios 1992). Two-tailed t-test was used to determine significance for means for heterozygosity and number of alleles per locus. One-way analysis of variance (ANOVA) was used to evaluate significant differences in enzyme activity between bivalve species and river locations (SAS 1985). Significance was inferred at $\alpha = 0.05$ and Duncan's multiple range test was used to determine means that were significantly different from highest enzyme activities for each species and each river. Pearson-Product correlation coefficients with a sequential-Bonferroni correction were used to investigate the interactions of several variables.

RESULTS

Genetics of the Populations

Electrophoretic analysis revealed varying polymorphism for the four species studied (for specific allele frequencies, see Johnson et al. 1998). The greatest level of polymorphism (P)

occurred in *A. plicata* (0.572) and *P. dombeyanus* (0.461) of the Cache River (Table 1). Cache River populations of *A. plicata* and *P. dombeyanus* exhibited downstream decreases in P and number of alleles per locus, whereas P values in *Q. pustulosa* and *Q. quadrula* of the White River did not exhibit consistent trends (Figs. 2,3). Several alleles for bivalve species in site B of the Cache River exhibited significant differences from those of other sites [e.g., the FUM-1 B allele ($X^2 = 4.85$, $p < .05$), the PGM-2 B allele ($X^2 = 6.333$, $p < .05$) in *A. plicata*, the MDH-2 B allele ($X^2 = 21.353$, $p < .05$), and the PGM-1 C allele ($X^2 = 13.937$, $p < .05$) in *P. dombeyanus*].

The average number of alleles per locus in the four species studied varied from 1.7 for both *Quadrula* species to 2.2 for *A. plicata* (Table 1). Significant downstream reductions in number of alleles per locus were identified for populations of *P. dombeyanus* and *A. plicata* from the Cache River ($p < .05$), with no trends identified for *Quadrula* populations for either species.

Genotype frequencies of the polymorphic loci were analyzed to determine which loci were in H-W equilibrium (Table 2). Most loci (0.67) were not in H-W equilibrium with most deviations (0.97) from expected due to heterozygote deficiencies. Heterozy-

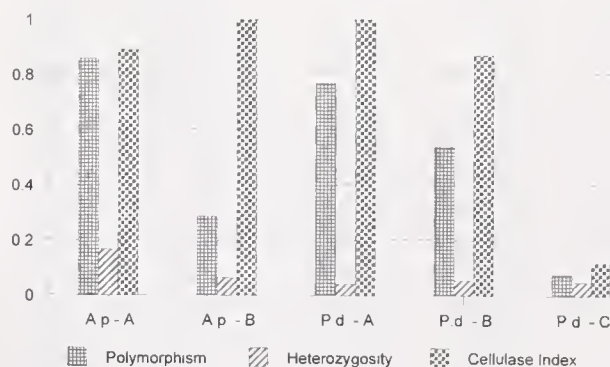


Figure 2. Polymorphism, heterozygosity, and cellulase index for *A. plicata* and *P. dombeyanus* in the Cache River (Sites A, B, C).

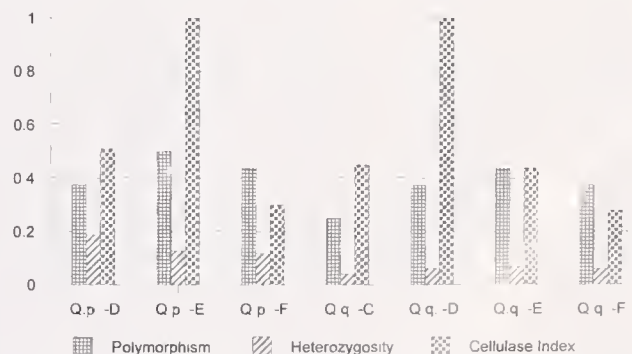


Figure 3. Polymorphism, heterozygosity, and cellulase index for *Q. pustulosa* and *Q. quadrula* in the Cache (Site C) and White (Sites D, E, F) rivers.

TABLE 2.

Number of polymorphic loci for populations of *A. plicata*, *P. dombeyanus*, *Q. pustulosa*, and *Q. quadrula*.

Site	Species			
	<i>A. plicata</i>	<i>P. dombeyanus</i>	<i>Q. pustulosa</i>	<i>Q. quadrula</i>
A	12 (10)	12 (12)	N/A	N/A
B	6 (4)	8 (5)	N/A	N/A
C	N/A	1 (0)	N/A	5 (3)
D	N/A	N/A	6 (4)	7 (6)
E	N/A	N/A	10 (3)	8 (5)
F	N/A	N/A	9 (3)	6 (5)

Number of loci demonstrating significant heterozygote deficiency (–) or excess from Hardy-Weinberg expectations as measured by “D” in parentheses ($P < .05$).

gote deficiencies were found in all populations studied with the exception of a single *P. dombeyanus* population which possessed one polymorphic locus out of 13 loci analyzed.

Quadrula pustulosa exhibited the highest mean heterozygosity ($H = 0.144$), followed successively by *A. plicata* ($H = 0.116$), *Q. quadrula* ($H = 0.058$), and *P. dombeyanus* ($H = 0.049$) (Table 1). *Quadrula pustulosa* had significantly greater heterozygosity than did *Q. quadrula* or *P. dombeyanus* ($p < .01$). Among the population heterozygosities were similar for *Q. quadrula* and *P. dombeyanus*, however, *A. plicata* and *Q. pustulosa* populations exhibited a significant decrease in H for downstream populations ($X^2 = 6.752$, $p < .01$; $X^2 = 8.711$, $p < .01$, respectively). Mean heterozygosity for the Ambleminae studied was 0.087 ± 0.048 .

Wright's weighted F -statistics were used to analyze the genetic structure of these populations (Table 3). The mean F_{ST} for pooled loci was significant for each of the four species. F_{ST} values showed moderate to high differentiation as defined by Hartl (1980), who described F_{ST} values of 0.05–0.15 as representative of moderately

differentiated populations and F_{ST} values of 0.15–0.25 for highly differentiated populations. Number of loci demonstrating significant differentiation among populations ranged from 5 for *Q. pustulosa* to 9 for *P. dombeyanus*. The greater number of loci demonstrating significant difference for *P. dombeyanus* is due in part to a single population (Site C) exhibiting a high degree of monomorphism (12 of 13 loci).

Cellulolytic activity measured in bivalves from the Cache River ranged from 911 (± 155) to 68 (± 12) U/g dry weight in *P. dombeyanus* and *Q. quadrula*, respectively. The only noticeable difference in enzyme activity between sites was in *P. dombeyanus* from site C, having significantly lower activity (12% of activity in upstream bivalves from site A (Table 4). Enzyme activity measured in *Q. quadrula* was also low, although we did not compare activity of any individuals from upstream sites for this particular species. Activities measured in *A. plicata* were similar at both sites A and B (177 ± 26 and 197 ± 35 , respectively).

Enzyme activities measured in the White River ranged from 150 ± 26 U/g dry weight for *Q. quadrula* to 25 ± 4 in *Q. pustulosa* (Table 4). Enzyme activity for individuals of sites E and F for *Q. quadrula* was significantly reduced compared with individuals from site D, whereas, enzyme activity of *Q. pustulosa* individuals sampled from sites D and F were significantly reduced compared with individuals from site E.

DISCUSSION

Several populations of *Q. quadrula* have been studied within the Mississippi River drainage system, including Wisconsin (Davis 1984) and Ohio (Berg et al. 1998). Ohio populations exhibited significantly greater polymorphism ($\bar{x} = 0.614$) than did Wisconsin ($\bar{x} = 0.357$) or Arkansas ($\bar{x} = 0.360$) populations ($p < .01$) (Table 5). Heterozygosity for *Q. quadrula* individuals of the present study (0.058) was dramatically lower than for previous individuals studied, with values one-half that of Wisconsin populations of the Mississippi River (0.112) and one-fourth that of Ohio

TABLE 3.

Summary of Wright's F -statistics for each polymorphic locus for populations of *A. plicata*, *P. dombeyanus*, *Q. pustulosa*, and *Q. quadrula* in Arkansas.

Locus	<i>A. plicata</i>		<i>P. dombeyanus</i>		<i>Q. pustulosa</i>		<i>Q. quadrula</i>	
	F_{IS}	F_{ST}	F_{IS}	F_{ST}	F_{IS}	F_{ST}	F_{IS}	F_{ST}
FUM-1	–0.601	0.119 ^c	0.472	0.036	N/A	N/A	N/A	N/A
FUM-2	1.000	0.096	1.000	0.022	0.650	0.036 ^a	–0.346	0.241 ^c
GOT-1	0.827	0.219 ^b	0.737	0.082 ^c	0.882	0.336 ^c	0.544	0.031
GOT-2	0.008	0.073 ^a	0.829	0.084 ^c	N/A	N/A	N/A	N/A
IDH-1	N/A	N/A	N/A	N/A	N/A	N/A	0.899	0.218 ^c
IDH-2	1.000	0.029	1.000	0.022	–0.021	0.016	–0.649	0.009
LDH-1	N/A	N/A	N/A	N/A	–0.042	0.036 ^b	N/A	N/A
MDH-1	–0.007	0.002	0.651	0.280 ^c	N/A	N/A	N/A	N/A
MDH-2	1.000	0.023 ^a	1.000	0.238 ^c	N/A	N/A	0.392	0.021
ME-1	0.978	0.003	1.000	0.090 ^c	0.597	0.141 ^c	0.433	0.022
ME-2	1.000	0.000	1.000	0.059 ^c	N/A	N/A	–0.415	0.230 ^c
MPI-1	0.935	0.197 ^c	0.697	0.455 ^c	0.706	0.494 ^c	–0.034	0.022
PGI-1	N/A	N/A	N/A	N/A	0.776	0.217 ^c	0.935	0.193 ^c
PGM-1	0.206	0.055	1.000	0.044 ^a	0.711	0.093 ^c	–0.175	0.057
PGM-2	1.000	0.171 ^c	0.415	0.037 ^c	0.669	0.074 ^c	0.309	0.146 ^c
Mean	0.510	0.082 ^a	0.738	0.121 ^c	0.618	0.160 ^c	0.046	0.108 ^b

^a $p < .05$; ^b $p < .01$; ^c $p < .001$.

TABLE 4.

Measured cellulase activity of Unionid bivalves collected from the Cache and White rivers, 1995.

Species	River Mile	Site	% Highest Activity/Species	Product \pm SE	n
Cache River					
<i>P. dombeyanus</i>	37	A	100	911 \pm 15	31
	36	B	87	793 \pm 439	9
	35	C	12 ^a	113 \pm 14	29
<i>A. plicata</i>	37	A	89	177 \pm 26	35
	36	B	100	197 \pm 35	36
<i>Q. quadrula</i>	35	C	45 ^a	68 \pm 12	36
White River					
<i>Q. quadrula</i>	63	D	100	150 \pm 26	36
	57	E	44 ^a	66 \pm 10	29
	48	F	28 ^a	41 \pm 7	24
<i>Q. pustulosa</i>	63	D	51 ^a	43 \pm 9	15
	57	E	100	85 \pm 57	11
	48	F	30 ^a	25 \pm 4	20

^a Significantly different from highest activity within individual species.

populations of the Ohio River drainage (0.24) ($p < .01$). Associated with this genetic decline is a reduction in the number of alleles per locus for Arkansas ($\bar{x} = 1.7$) versus Ohio ($\bar{x} = 2.1$) populations of *Q. quadrula* ($p < .05$).

Populations of *A. plicata* have also been studied in Ohio by Berg et al. (pers. comm.). Polymorphism, heterozygosity, and number of alleles per locus for Ohio and Arkansas populations of *A. plicata* were similar (0.575 versus 0.572; 0.11 versus 0.12; 2.0 versus 2.2, respectively).

Genetic diversity of the Ambleminae studied was comparable to that of other Ambleminae (Table 5). Polymorphism in the four species studied ranged from 0.360 to 0.572, with a mean of 0.458 ± 0.076 , insignificantly different than mean p values of 0.382 ± 0.154 for Ambleminae of past studies. Direct-count heterozygosity values and number of alleles per locus were also similar to those observed by other researchers (0.092 ± 0.040 versus 0.107 ± 0.056 ; 1.8 ± 0.2 versus 1.9 ± 0.5 , respectively). H and P values for Ambleminae (all studies) were significantly greater than for Margaritiferidae and Anodontinae ($p < .01$).

Hardy-Weinberg disequilibrium has been identified for loci in many bivalve populations (Milkman and Beaty 1970; Hornbach et al. 1980; Adamkewicz et al. 1984; Stiven and Alderman 1992), yet not of the magnitude as in the present study. Many explanations have been offered for heterozygote deficiencies, including bottleneck effects, Wahlund effect, the presence of null alleles, linkage disequilibrium, inbreeding and selection (Singh and Green 1984).

The combined effects of harvesting and habitat modification could be producing a bottleneck effect, where genetic drift is serving as a dominant force in gene frequency changes of these populations (Nei et al. 1975). Bottleneck effects have been demonstrated to reduce heterozygosity for hundreds of thousands of years following population recovery (Nei et al. 1975). Historic quantitative data are not available, but Christian (1995) reported a dramatic reduction in numbers of bivalve beds, and anecdotal reports by shellers have described a precipitous decrease in numbers of bivalves available for harvest (K. C. Ward, E. Kohal, J. T. Easter, pers. comm.). If bottlenecks were indeed occurring, genetic diversity (P , number of alleles/locus, and H) should be reduced

relative to other populations (Leberg 1992). This is indeed the case for *Q. quadrula*. As stated previously, H , P , and number of alleles per locus were significantly less for Arkansas versus Ohio populations of *Q. quadrula* (Berg et al. 1998). H was significantly reduced relative to Wisconsin populations; no significant differences were identified for P and number of alleles per locus (Davis 1984). No significant differences were found for genetic diversity of Arkansas and Ohio populations of *A. plicata* (Berg, pers. comm.). No genetic diversity studies were available for comparisons of *P. dombeyanus* and *Q. pustulosa*.

Null alleles represent the loss of enzyme activity for multimeric enzymes (e.g., dimers, trimers, tetramers) in heterozygotes due to structural instability (Milkman and Beaty, 1970; Koehn and Eanes, 1978). Zouros and Foltz (1984) successfully argued against the presence of null alleles as determinants of heterozygote deficiency in bivalves. No relationship was found in the present study between the number of subunits in the functional enzyme and the degree of H - W disequilibrium ($p < .75$).

Zouros and Foltz (1984) considered the existence of independently reproducing subgroups (Wahlund effect) as a viable explanation for highly variable bivalve populations. Bivalve bed sizes for the present study ranged from 200 to 780 m² (Christian, 1995); dispersal mechanisms should adequately prevent the formation of subpopulations within these moderately sized bivalve beds.

Inbreeding has also been proposed as an explanation for heterozygote deficiency (Hornbach et al. 1980). Koehn et al. (1971) stated that if inbreeding is indeed occurring, heterozygote deficiency should be consistent for all polymorphic loci. Inbreeding may be occurring, particularly for *P. dombeyanus*, as each locus exhibited heterozygote deficiency. Very few loci were not subject to heterozygote deficiency (e.g., GOT-2 and PGM-1 for *A. plicata*; MDH-2, MPI-1, PGM-2 in *Q. pustulosa*; IDH-2 for *Q. quadrula*).

Nei et al. (1975) have previously identified significant positive correlations of population size to heterozygosity values, with inbreeding enhanced in smaller populations. No significant correlation was observed between effective population size as determined by Christian (1995) and heterozygosity ($r_s = 0.14$; $p < .62$).

Extrinsic factors may be responsible for the high degree of heterozygote deficiency within these bivalve populations. Low heterozygosity has been associated with a reduction in resistance to pollutants (Nevo et al. 1986), although contradictory data exist as to the presence of a selective effect of toxicants on heterozygotes (Nevo et al. 1981; Lavie and Nevo 1986). Toxicants appear to exert a greater selective effect on specific loci as compared with overall heterozygosity (Diamond et al. 1991; Schleuter et al. 1995).

Site B on the Cache River represents a low-lying catch basin for nonpoint agricultural runoff, which would include fertilizers, herbicides, pesticides, and defoliants (The runoff and sediment have not been analyzed to date.). If a wide range of toxicants introduced into the Cache River were exerting a selective influence on specific genotypes, then a lower variability of genotypes would be predicted, which was consistent with our findings. Other researchers have documented alleles differentially sensitive to specific toxicants in a laboratory setting (Lavie and Nevo 1986; Lavie and Nevo 1988; Diamond et al. 1991; Gillespie and Guttman 1989; Schleuter et al. 1995). However, this differential sensitivity is not consistent for all enzyme systems (Diamond et al. 1991). Schleuter et al. (1995) proposed that different environmental stressors have different effects on specific loci.

TABLE 5.
Genetic diversity as measured by heterozygosity (H), polymorphism (P), and alleles per locus (A/L) for freshwater bivalves of North America.

Taxa	H	P	A/L	Reference
Family Margaritiferidae				
<i>Margarita margaritifera</i>	0.030	0.142	N/A	Davis et al. 1981
Family Unionidae				
Subfamily Anodontinae				
<i>Alasmidonta undulata</i>	0.013 (0.002)	0.071 (0.000)	N/A	Kat and Davis 1983
<i>Anodonta cataracta</i>	0.028 (0.006)	0.113 (0.039)	1.1 (0.0)	Kat 1983a
<i>A. c. fragilis</i>	0.081 (0.004)	0.237 (0.041)	1.1 (0.0)	Kat 1983a
<i>A. gibbosa</i>	0.106	0.285	1.3	Kat 1983ab
<i>A. implexata</i>	0.061 (0.006)	0.357 (0.000)	1.4 (0.0)	Kat 1983a
Subfamily means	0.058 (0.034)	0.213 (0.106)	1.2 (0.1)	
Subfamily Ambloleminae				
<i>Amblolema plicata</i>	0.110	0.575	2.0	Berg pers. comm.
<i>A. plicata</i>	0.116 (0.052)	0.572 (0.286)	2.1 (0.5)	Present study
<i>Elliptio arcata</i>	0.112	0.429	N/A	Davis 1984
<i>E. buckleyi</i>	0.105	0.285	N/A	Davis 1984
<i>E. cistelliformis</i>	0.080	0.357	N/A	Davis et al. 1981
<i>E. congaraea</i>	0.187	0.357	N/A	Davis 1984
<i>E. complanata</i>	0.145 (0.019)	0.482 (0.106)	N/A	Davis et al. 1981
<i>E. complanata</i>	0.060 (0.011)	0.425 (0.051)	2.5 (0.4)	Kat and Davis 1983
<i>E. complanata</i>	0.130	0.50	2.3	Davis 1983
<i>E. complanata</i>	0.169	0.818	3.4	Stiven & Alderman 1992
<i>E. crassidens</i>	0.171 (0.033)	0.453 (0.033)	N/A	Davis 1984
<i>E. dilatata</i>	0.106	0.285	N/A	Davis 1984
<i>E. fisheriana</i>	0.085	0.286	N/A	Davis et al. 1981
<i>E. folliculata</i>	0.100	0.286	N/A	Davis et al. 1981
<i>E. ictarina</i>	0.105 (0.027)	0.333 (0.119)	N/A	Davis et al. 1981
<i>Elliptio lanceolata</i>	0.049	0.142	N/A	Davis et al. 1981
<i>E. mcMichaeli</i>	0.160 (0.010)	0.500 (0.000)	N/A	Davis 1984
<i>E. shepardiana</i>	0.077	0.214	N/A	Davis 1981
<i>E. waccamawensis</i>	0.099	0.357	N/A	Davis et al. 1981
<i>Fusconaia flava</i>	0.110	0.290	1.6	Davis 1983
<i>F. sucissa</i>	0.081	0.428	N/A	Davis et al. 1981
<i>Lampsilis cariosa</i>	0.070	0.430	N/A	Kat and Davis 1983
<i>L. cariosa</i>	0.286 (0.024)	0.757 (0.086)	2.4 (0.1)	Stiven & Alderman 1992
<i>L. fullerkati</i>	0.169	0.455	1.9	Stiven & Alderman 1992
<i>L. ochracea</i>	0.044 (0.007)	0.262 (0.034)	N/A	Kat 1983b
<i>L. ochracea</i>	0.049 (0.004)	0.286 (0.000)	N/A	Kat and Davis 1983
<i>L. radiata</i>	0.028 (0.016)	0.309 (0.067)	N/A	Kat 1983b
<i>L. radiata</i>	0.022 (0.015)	0.228 (0.114)	1.6 (0.5)	Kat and Davis 1983
<i>L. r. conspicua</i>	0.138	0.727	2.2	Stiven & Alderman 1992
<i>L. r. radiata</i>	0.162	0.364	1.5	Stiven & Alderman 1992
<i>L. splendida</i>	0.059	0.428	N/A	Kat 1983b
<i>L. teres</i>	0.056	0.286	N/A	Davis et al. 1981
<i>Leptodea ochracea</i>	0.076 (0.025)	0.319 (0.046)	1.8 (0.2)	Stiven & Alderman 1992
<i>Megalania boykiniana</i>	0.022	0.143	N/A	Davis 1984
<i>Plectomerus dombeyanus</i>	0.040 (0.006)	0.461 (0.288)	1.8 (0.6)	Present study
<i>Quadrula pustulosa</i>	0.144 (0.031)	0.438 (0.051)	1.6 (0.2)	Present study
<i>Q. quadrula</i>	0.112	0.357	1.6	Davis 1984
<i>Q. quadrula</i>	0.240 (0.026)	0.614 (0.069)	2.1 (0.3)	Berg et al. 1998
<i>Q. quadrula</i>	0.058 (0.010)	0.360 (0.068)	1.6 (0.2)	Present study
<i>Quincuncina infucata</i>	0.080	0.290	1.6	Davis 1983
<i>Unio merus carolinianus</i>	0.090	0.210	1.3	Davis 1983
<i>U. declivis</i>	0.120	0.210	1.4	Davis 1983
<i>U. exultus</i>	0.110	0.430	1.5	Davis 1983
Subfamily means	0.107 (0.056)	0.382 (0.154)	1.9 (0.5)	

In further analyzing the two species studied for the Cache River, heterozygosity declined dramatically for Site B as compared with Site A in *Amblyema plicata*. The observed reduced cellulolytic activity in *A. plicata* for both sites A and B suggests that individual bivalve condition was responding to some ongoing perturbant at the time of collection. For *P. dombeyanus*, heterozygosity was constant for sites A, B, and C; however, bivalves from Site C had significantly lower cellulase activity as compared with sites A and B. The cellulase index integrates a continuum of responses (e.g., behavior-mediated, reduction in efficiency of utilizing available food) to chronic exposures. At times a quantitative biomarker such as this is made more revealing by examining the variance of the measurement for a given site. Early seasonal-associated perturbants (chemical application and runoff) may have accumulated in the sediment. Cellulase activity measured during late summer, low flow, may have been affected by these earlier-season perturbants due to food source accumulation. Cellulase activity studies have focused on unique species responses that are not always predictable from measurement of ambient water concentrations (Farris et al. 1988). For example, Cherry et al., (1988) measured reduced bivalve enzyme activity with tissue metal accumulations that were manifested at site-specific growth reduction. These effects were attributed to uptake from algal food sources when water metal concentrations were below detection. An outfall pipe at St. Charles, AR, dumps surface runoff immediately upstream of Site E and was therefore selected as a sampling site that could provide better resolution between environmental cause and physiological effect. Again, sediment and surface runoff has not been analyzed as yet for composition. No genetic differences were identified, as polymorphism, alleles per locus, and heterozygosity were similar between populations for both species of *Quadrula* within the white River. However, *Q. quadrula* individuals possessed significantly less enzyme activity within Sites E and F, whereas the cellulolytic activity was significantly greater for the middle population (Site E) of *Q. pustulosa* than for the other two populations.

We investigated the cellulolytic activity of individuals within populations and species as a function of heterozygosity, with a hypothesis that there would be a heterozygote vigor. This has been demonstrated in several other studies involving metabolism and growth rates (Koehn et al. 1976; Koehn and Shumway 1982; Garton et al. 1984; Singh and Green 1984; Koehn et al. 1988). Correlation coefficients very closely approximated zero for almost every sample (Table 6).

TABLE 6.
Relationship of cellulolytic activity to heterozygosity for populations of Unionidae within the Cache and White rivers, Arkansas.

Species	Site	N	Corr. Coeff.
Cache River			
<i>P. dombeyanus</i>	A	31	0.070
	B	9	0.859
	C	29	-0.089
<i>A. plicata</i>	A	35	-0.072
	B	36	-0.191
<i>Q. quadrula</i>	C	36	0.102
White River			
<i>Q. pustulosa</i>	A	15	0.187
	B	11	0.442
	C	20	0.055
<i>Q. quadrula</i>	D	36	-0.024
	E	29	0.035
	F	24	0.236

No significant correlations were identified.

Since cellulase activity reflects functional responses to river autotrophic production as well as a host of perturbant effects, one seasonal examination was insufficient to draw any conclusions related to site effects. Repeated bimonitoring for these sites could relate the observed species and site variances to physiological stress more appropriately. Seasonal study could provide the necessary biomonitoring and chemical monitoring to evaluate the composition of the runoff and sediment before introduced toxicants can be posed as a selective mechanism explaining genetic and physiological changes in downstream Cache River bivalve populations.

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CORRELATION BETWEEN MATING SYSTEM AND DISTRIBUTION OF GENETIC VARIATION IN *UTTERBACKIA* (BIVALVIA: UNIONIDAE)

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ABSTRACT Variation in mating systems (e.g., dioecy versus hermaphroditism, self-fertilization versus cross-fertilization) has been shown to affect the distribution of genetic variation in plants. However, the paucity of this type of variation in closely related taxa has hampered similar evaluations in animals. The freshwater bivalve genus *Utterbackia* (Unionidae: Anodontinae), currently comprising three nominal and one undescribed species, contains gonochoric (dioecious) as well as hermaphroditic species and thus is a model animal system for examining mating system evolution and its effects on the distribution of genetic variation. Comparisons of the level of within- and among-population allozymic variation (at 9 putative genetic loci) in the simultaneous hermaphrodites *Utterbackia imbecillis* (23 populations, 331 individuals) and *U. "imbecillis"* (4 populations, 51 individuals), with those of the gonochoric *U. peggyae* (7 populations, 99 individuals) and *U. peninsularis* (6 populations, 77 individuals) allowed inferences to be made regarding (1) the mating system of *U. imbecillis* and *U. "imbecillis"* and (2) the population genetic structure of these four species. The low levels of within-population variation and marked heterozygote deficiency observed in *U. imbecillis* and *U. "imbecillis"* relative to that in *U. peggyae* and *U. peninsularis*, suggest that there is a high degree of self-fertilization in both hermaphroditic species. However, the among-population variation in the level of heterozygote deficiency (Selander D range: -0.181 to -1.000) is consistent with the hypothesis that the relative amounts of cross-fertilization and self-fertilization vary among populations of *U. imbecillis* and *U. "imbecillis"*. The hypothesis of high levels of self-fertilization in *U. imbecillis* and *U. "imbecillis"* is consonant with the presumed high colonization potential of the former species. The estimates of F_{ST} obtained for the four species of *Utterbackia* suggest a very high level of among-population genetic differentiation (mean F_{ST} range: 0.218–0.818). This observation is quite unexpected for an able colonizer such as *U. imbecillis* (mean F_{ST} = 0.818) unless detailed knowledge of this species' mating system is considered. The combined impact of self-fertilization and founder events in the hermaphroditic *Utterbackia* species likely potentiates among-population genetic differentiation which increases F_{ST} values. These results suggest that the mating system and distribution of genetic variation in unionid populations should be carefully evaluated prior to the enactment of conservation initiatives.

KEY WORDS: Simultaneous hermaphroditism, gonochorism, self-fertilization, cross-fertilization, heterozygote deficiencies, allozymes, genetic variation

INTRODUCTION

Taxa that display variation in mating systems are currently under intense scrutiny because of their utility in addressing ques-

tions of geographic variation and adaptation (Charnov 1982, Rick 1988, Wyatt 1988, Waller 1993, Waser 1993, Barrett and Harder 1996, Reynolds 1996). Mating system variation at relatively low taxonomic levels (among both congeneric species and conspecific populations) is especially useful since the factors responsible for the mating system transitions may still be operative. Furthermore, explicit hypotheses of phylogenetic relationships may permit an assessment of the directionality of character state change in mating systems. Therefore, the presence of gonochorism (dioecy) and simultaneous hermaphroditism within the freshwater bivalve genus *Utterbackia* (Baker 1927) (Bivalvia: Unionidae: Anodontinae),

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combined with an explicit hypothesis of phylogenetic relationships available for the group (Hoeh et al. 1995), renders this taxon a model system for evaluating the forces responsible for mediating mating system evolution. However, the current lack of understanding regarding the details of the fertilization system (e.g., cross-fertilizing versus self-fertilizing) within the hermaphroditic species of *Utterbackia* impedes our ability to evaluate the relative importance of potential mediating forces.

Currently, *Utterbackia* consists of three nominal species—*U. imbecillis* (Say 1829), *U. peggyae* (Johnson 1965), and *U. peninsularis* (Bogan and Hoeh 1995) and one undescribed species (*U. "imbecillis"*) (see Hoeh et al. 1995). The geographic distribution of *U. imbecillis* includes much of the eastern half of the USA, and portions of southern Canada and northern Mexico, and *U. peggyae*, *U. peninsularis*, and *U. "imbecillis"* are restricted to the drainages flowing through the panhandle of Florida, peninsular Florida, and southern Atlantic Slope regions, respectively, of the southeastern USA (Hoeh et al. 1995). The four species within *Utterbackia* are ecologically similar, inhabiting lentic and lotic habitats with relatively low current velocities (Baker 1928, Johnson 1965, Cummings and Mayer 1992). As for most unionids, *Utterbackia* larvae are likely short-duration, obligate parasites of freshwater fish (Fuller 1974). However, host fish data are currently available for only *U. imbecillis* (Hogarth 1992). A recent cladistic analysis of the genus (Hoeh et al. 1995) resulted in the following phylogenetic hypothesis: [*U. imbecillis sensu lato*, *U. peninsularis*, *U. peggyae*]. Together with a paleogeographic hypothesis of Late Cenozoic sea level fluctuations in the southeastern USA, this analysis allowed the inference that the *U. imbecillis s.l.* initially diverged from the *U. peninsularis* lineage approximately 5 million years ago in freshwater drainages of the southeastern USA (Hoeh et al. 1995).

A significant difference in sexuality exists between *Utterbackia imbecillis s.l.* and both *U. peggyae* and *U. peninsularis*. Like the majority of unionid bivalves, *U. peggyae* and *U. peninsularis* are gonochoric whereas *U. imbecillis* and *U. "imbecillis"* are simultaneous hermaphrodites (i.e., produce mature oocytes and spermatozoa concurrently; Sterki 1998a, Sterki 1998b, Ortmann 1910, Ortmann 1911, Allen 1924, Baker 1927, Baker 1928, van der Schalie 1970, Heard 1975, Kat 1983). Based on a parsimony criterion, it was hypothesized that a single transition, from gonochorism to simultaneous hermaphroditism, occurred in the ancestor of the *U. imbecillis s.l.* lineage subsequent to its divergence from the *U. peninsularis* lineage (Hoeh et al. 1995). This hypothesis suggests that simultaneous hermaphroditism is a relatively recently evolved form of sexuality derived from a gonochoric ancestral taxon.

Variability in the mating systems of simultaneous hermaphroditic taxa (i.e., from obligate cross-fertilizing to obligate self-fertilizing) is well known in plants (Allard 1975, Stebbins 1970, Stebbins 1974, Jain 1976, Schoen 1982, Schemske and Lande 1985, Holtsford and Ellstrand 1990, Waller 1993, Barrett and Harder 1996) and has been documented in mollusks (Tompa et al. 1984, McCracken and Selander 1980, Foltz et al. 1984, O'Foighil and Eernisse 1987, O'Foighil and Eernisse 1988, Eernisse 1988). The following data suggest that this type of mating system variability exists within *U. imbecillis*.

Extrapolating from relative cross-sectional areas of stained paraffin sections from a standard position in the visceral mass, Kat (1983) reported a bimodal distribution of testicular to ovarian tissue volume ratios among 15 populations of *Anodonta imbecilis*

(sic) (*Utterbackia imbecillis*; it is possible that several of these populations actually represented *U. "imbecillis"*). Among Kat's "central geographic range" populations, relatively low density populations from running water had significantly higher average ratios of male to female gonadal tissue than did high density populations from standing water. Given the current understanding of the distributions of *U. imbecillis* and *U. "imbecillis"*, it seems unlikely that this particular result can be attributed to species-specific gonadal allocations between *U. imbecillis* and *U. "imbecillis"*.

Kat's (1983) preferred explanation for the bimodality phenomenon was that selection adjusted sperm production to facilitate efficient cross-fertilization in local populations. Under this cross-fertilization efficiency hypothesis, relatively high density, low current velocity populations would require smaller amounts of spermatozoa to effect efficient cross-fertilization than would low density, high current velocity populations. Kat also suggested that sperm limitation could reduce fecundity in the latter situation. However, unless it is assumed that *U. imbecillis* only exists in extremes of both population density and current velocity, this hypothesis does not explain the bimodal nature of the data. Alternatively, sex allocation theory predicts a strong ovarian bias in the gonad of selfing hermaphrodites and an approximately equal amount of testicular and ovarian tissue in cross-fertilizing hermaphrodites (Charnov 1982). Thus, Kat's (1983) populations with relatively high ratios of male to female gonadal tissue may be predominantly cross-fertilizing whereas those with relatively low ratios may be predominantly self-fertilizing. This hypothesis was noted by Kat (1983) but rejected because of lack of substantiating data.

If self-fertilization has played a role in the genesis of the variation in gonadal allocation observed in *Utterbackia imbecillis*, one could predict that this species' population genetic structure would be very different from that of the gonochoric (i.e., obligately cross-fertilizing) *U. peggyae* and *U. peninsularis*. Prolonged selfing should result in a decay of heterozygosity (Crow and Kimura 1970). Even though total allozymic monomorphism is not unknown in gonochoric species, it is the expected consequence of a prolonged selfing regime combined with genetic drift (Wright 1969). Thus, the occurrence of considerable within-population genetic diversity is contrary to the expectations of prolonged selfing in combination with drift (Hamrick et al. 1979, Schoen 1982, Loveless and Hamrick 1984, Hamrick and Godt 1990), whereas selfing is but one of several mechanisms that might produce highly homozygous strains.

Given the above, with respect to within-population genetic structure, a predominantly self-fertilizing *U. imbecillis* would be expected to have a much lower level of variation than the obligately cross-fertilizing *U. peggyae* and *U. peninsularis*. Furthermore, the observed frequency of heterozygotes in a predominantly self-fertilizing *U. imbecillis* population should be much lower than the frequencies predicted by Hardy-Weinberg equilibria (Crow and Kimura 1970). Additionally, predominantly self-fertilizing species would likely have higher among-population levels of genetic differentiation than would cross-fertilizing species (Levin 1978, Loveless and Hamrick 1984, Holtsford and Ellstrand 1989, Hamrick and Godt 1990). Founder effects, due to a small number (often one?) of homozygous colonizing individuals, are likely responsible for the generality of this phenomenon among self-fertilizing lineages.

In order to evaluate Kat's (1983) cross-fertilizing/self-fertilizing hypothesis as a possible explanation for the observed

gonadal allocation in *U. imbecillis*, the present study analyzed a total of 40 populations of *Utterbackia* for allozyme variation at nine presumptive loci. The patterns of within-population genetic variation obtained from the allozyme analyses were used to categorize the mating system of 23 populations of *U. imbecillis* and four populations of *U. "imbecillis."* Genetic evidence consistent with the hypothesis of high levels of self-fertilization for Kat's (1983) low testicular: ovarian tissue ratio *U. imbecillis* populations would provide sufficient justification to reject Kat's cross-fertilization efficiency hypothesis for a more parsimonious alternative. Among-population patterns of genetic variation were also examined to allow for a more complete evaluation of potential correlates between population genetic structure and mating system in *Utterbackia*. Furthermore, the observed population genetic structures within *Utterbackia* were evaluated with regard to potential conservation initiatives.

MATERIALS AND METHODS

In an attempt to secure adequate sampling of the genetic variability within *Utterbackia*, 23 populations of *U. imbecillis* (331 individuals), four populations of *U. "imbecillis"* (51 individuals), seven populations of *U. peggyae* (99 individuals), and six populations of *U. peninsularis* (77 individuals) were sampled. Sample size and locality information are presented in Appendix I. A diligent effort was made to collect all specimens at a site. Voucher specimens, representing *U. peggyae*, *U. peninsularis*, *U. imbecillis*, and *U. "imbecillis,"* have been deposited in the Museum of Zoology, University of Michigan (Mollusk Division #253578, 253579, 253580, and 253581, respectively). Soon after specimen collection, non-gravid gill tissues were excised and cleaned of macroscopic parasites and debris, frozen in liquid nitrogen, and stored at -70°C . Tissues were homogenized with a glass pestle in 1.5 ml microcentrifuge tubes. The gill tissues contained sufficient water to eliminate the need for homogenization buffer. The resultant homogenates were centrifuged at $13,605 \times g$ for 10 min at 4°C .

Horizontal starch gel electrophoresis (12% starch gels; 51 g Connaught starch in 425 ml of gel buffer) was used to detect electromorphs at nine polymorphic, putative genetic loci using four buffer systems (Appendix II). Stain recipes followed Shaw and Prasad (1970), Siciliano and Shaw (1976), and Murphy et al. (1990).

Data analyses were carried out using the BIOSYS-1 (Swofford and Selander 1981) computer program. In order to evaluate the patterns of within-population genetic variation, the mean number of alleles per locus, number of polymorphic loci, expected and observed heterozygosities, departure of variable loci from H-W expectations (significance test using exact probabilities; significance criterion, $p \leq .05$), and heterozygote deviation at variable loci relative to H-W expectations (Selander's D, 1970; [Ho-He]/He) were calculated for each population. Selander's D can range from -1 (no heterozygous individuals) to $+1$ (all individuals heterozygous) with zero being the expected value if the population is in H-W equilibrium. Mann-Whitney U tests were employed to evaluate the significance of differences between the gonochoric and hermaphroditic species for the above within-population descriptive statistics. Among-population patterns of genetic variation were evaluated using Wright's (1978) F_{ST} .

RESULTS

A summary of the within-population genetic variation for the 40 populations of *Utterbackia* examined is presented in Table 1.

The multilocus genotypes generated for the 558 individuals are available upon request from the senior author. Of the 23 *U. imbecillis* populations, 13 were monomorphic at all nine loci. For the remaining 10 populations, the number of polymorphic loci ranged from one to three. Two of the four *U. "imbecillis"* populations were monomorphic at all assayed loci, and the remaining two populations were each polymorphic at a single locus. The number of polymorphic loci per population in *U. peggyae* and *U. peninsularis* ranged from 1 to 3 and 1 to 4, respectively. The mean number of polymorphic loci per population for *U. imbecillis*, *U. "imbecillis,"* *U. peggyae*, and *U. peninsularis* was 0.74, 0.50, 2.57, and 2.00, respectively. The mean number of alleles per locus per population was 1.08 for *U. imbecillis*, 1.06 for *U. "imbecillis,"* 1.38 for *U. peggyae*, and 1.26 for *U. peninsularis*. Mann-Whitney U tests detected no significant differences in either number of polymorphic loci per population or mean number of alleles per locus between *U. imbecillis* and *U. "imbecillis"* and between *U. peggyae* and *U. peninsularis*. However, there was a significant difference in both number of polymorphic loci per population ($U = 44.5$, $p = .0002$) and mean number of alleles per locus ($U = 35.5$, $p < .0001$) between the combined gonochoric and combined hermaphroditic species groupings.

Eleven of 17 (65%) and 1 of 2 (50%) of the population by polymorphic locus pairs were not within H-W expectations for *U. imbecillis* and *U. "imbecillis,"* respectively. In contrast, only 1 of 18 (6%) and 1 of 12 (8%) were not for *U. peggyae* and *U. peninsularis*, respectively. The range of heterozygote deviation scores (Selander's D, 1970) for the genetically variable populations of *U. imbecillis*, *U. "imbecillis,"* *U. peggyae*, and *U. peninsularis* was -1.000 to -0.181 , -1.00 to -0.632 , -0.177 to 0.197 , and -0.120 to 0.364 , respectively. The mean heterozygote deviation score for *U. imbecillis* was -0.661 , for *U. "imbecillis"* -0.816 , for *U. peggyae* 0.031 , and for *U. peninsularis* 0.032 . Mann-Whitney U tests detected no significant differences in either number of loci with significant departures from H-W expectations or heterozygote deviation scores between *U. imbecillis* and *U. "imbecillis"* and between *U. peggyae* and *U. peninsularis*. However, there was a significant difference in both number of loci with significant departures from H-W expectations ($U = 35.0$, $p = .0193$) and heterozygote deviation scores ($U = 0$, $p < .0001$) between the combined gonochoric and combined hermaphroditic species groupings.

Among-population genetic differentiation was relatively high in the four species of *Utterbackia*. Mean F_{ST} for *U. imbecillis*, *U. "imbecillis,"* *U. peggyae*, and *U. peninsularis* was 0.818 (based on 5 loci), 0.218 (1 locus), 0.348 (6 loci), and 0.600 (5 loci), respectively. No clear pattern relating mating system and levels of among-population genetic differentiation emerged from this analysis. For example, the two hermaphroditic species, *U. imbecillis* and *U. "imbecillis,"* had both the highest and lowest mean F_{ST} values, respectively, for the genus. However, the relatively low mean F_{ST} value for *U. "imbecillis"* (0.218) may be due to both the limited number of populations (four) and the few variable loci (one). Overall, the relatively high F_{ST} values observed in *Utterbackia* may be due, in part, to the sampling design of the present study. Most of the drainages represented contained a single sampled population of each species. Expanding the within-drainage sampling for each species would likely reduce their global F_{ST} values.

Overall, both individuals and populations of the simultaneously hermaphroditic *U. imbecillis* and *U. "imbecillis"* had less genetic variation than the gonochoric *U. peggyae* and *U. peninsularis*.

APPENDIX 1.

APPENDIX 1.

Locality information and sample sizes for the 40 populations of *Utterbackia* examined in this study.

Utterbackia imbecillis (n = 331)

- 1) ACi-Apalachicola River, below Lake Seminole, Chattahoochee, Gadsden Co., FL (n = 33)
- 2) BHCi-Ocmulgee River, at the Ben Hill/Coffee Co. line boat ramp, GA (n = 11)
- 3) CACi-C100A canal, Miami, Dade Co., FL (n = 4)
- 4) CCLi-Lake Corpus Christi, at Lake Corpus Christi State Park, San Patricio Co., TX (n = 7)
- 5) CDi-Cedar River, below Wiggins Lake, Gladwin Co., MI (n = 32)
- 6) DCLi-Deep Creek, at US Route 258, Edgecombe Co., NC (n = 5)
- 7) DRLi-mouth of Dead River Lake, off of the Pascagoula River, Jackson Co., MS (n = 7)
- 8) GALi-Pond at Suntree Country Club, Brevard Co., FL (n = 33)
- 9) GLCi-Gantt Lake, off of US Route 29, Covington Co., AL (n = 14)
- 10) KLTi-Kentucky Lake, at Paris Landing State Park, Henry Co., TN (n = 18)
- 11) KRK8i-Kokosing River, below Knox Lake, Knox Co., OH (n = 7)
- 12) KRK9i-Kankakee River, at US Route 45, Kankakee, Kankakee Co., IL (n = 6)
- 13) KRMi-Kankakee River, Momence, Kankakee Co., IL (n = 4)
- 14) LCHi-Lake Cass, Hillsborough Co., FL (n = 16)
- 15) LRGi-Little River, at FL Route 12, Gadsden Co., FL (n = 5)
- 16) LTCi-Ochlockonee River, Lake Talquin, Coe's Landing, Leon Co., FL (n = 12)
- 17) MCi-Mill Creek, below Starve Hollow Lake, Jackson Co., IN (n = 12)
- 18) MCWi-Mantua Creek, at Lambs Road bridge, Pitman, Gloucester Co., NJ (n = 7)
- 19) OSFi-Oklawaha River, at FL Route 314, Marion Co., FL (n = 24)
- 20) PCi-Pickering Creek, at PA Route 23, Chester Co., PA (n = 12)
- 21) PRWi-Pearl River, at Walkiah Bluff, Pearl River Co., MS (n = 4)
- 22) SRi-Canal off of the Suwannee River, at Dilger's Campground, Dixie Co., FL (n = 34)
- 23) UPPi-Pond off of Poinciana Blvd., Polk Co., FL (n = 24)

U. imbecillis (n = 51)

- 1) CRHi-Combahee River, at US Route 17A, Hampton Co., SC (n = 11)
- 2) FPCi-Fisher's Pond, Mt. Pleasant, Cabarrus Co., NC (n = 20)
- 3) ORTi-Ottoopec River, at GA Route 147, Tattnall Co., Ga (n = 7)
- 4) SRGi-Saluda River, at Saluda Dam Road, Pickens Co., SC (n = 13)

U. peggyae (n = 99)

- 1) ACHp-Attapulgus Creek, at FL Route 159, Gadsden Co., FL (n = 16)
- 2) CSLp-Chumuckla Springs Lake, Santa Rosa Co., FL (n = 15)
- 3) HCCp-Holmes Creek, at US Route 90, Holmes Co., FL (n = 8)
- 4) LRGP-Little River, at FL Route 12, Gadsden Co., FL (n = 16)
- 5) LTCp-Ochlockonee River, Lake Talquin, Coe's Landing, Leon Co., FL (n = 15)
- 6) SHFp-sink hole, at Florida Caverns State Park, Jackson Co., FL (n = 19)
- 7) YRCp-Yellow River, at US Route 90, Okaloosa Co., FL (n = 10)

continued

U. peninsularis (n = 77)

- 1) GCPs-Gator Creek, at FL Route 471, Polk Co., FL (n = 14)
- 2) HRHs-Hillsborough River, at FL Route 579, Hillsborough Co., FL (n = 13)
- 3) HRPs-Hillsborough River, at FL Route 39, Pasco Co., FL (n = 5)
- 4) NRBs-New River, at County Route 231, Bradford Co., FL (n = 5)
- 5) RCAs-Rocky Creek, at FL Route 235, Alachua Co., FL (n = 20)
- 6) SRs-canal off of the Suwannee River, at Dilger's Campground, Dixie Co., FL (n = 20)

Extreme heterozygote deficits were commonly observed in both *U. imbecillis* and *U. "imbecillis"*. Neither *U. peggyae* nor *U. peninsularis* exhibited significant wide-scale heterozygote deficiencies similar to those observed in many gonochoric marine bivalve species (Singh and Green 1984, Zouros and Foltz 1984, Gaffney et al. 1990).

DISCUSSION

Mating Systems in Utterbackia imbecillis s.l.

Heterozygote deficiencies in marine bivalves have been attributed to (1) inbreeding, (2) inadvertently sampling multiple differentiated subpopulations at a single locality (Wahlund effect; Wahlund 1928), (3) selection against heterozygotes, (4) null alleles, (5) aneuploidy, and (6) molecular imprinting (Gaffney et al. 1990). The relatively large number of populations with substantial heterozygote deficiencies noted in the present study makes it unlikely that sampling differentiated subpopulations is the major explanation for the observed heterozygote deficiencies in *U. imbecillis* and *U. "imbecillis"*. Furthermore, the absence of substantial heterozygote deficiencies in the congeneric and ecologically similar, but gonochoric, *U. peggyae* and *U. peninsularis* does not support this explanation. Since particular populations of *U. imbecillis* had multiple polymorphic loci displaying significant heterozygote deficiencies (populations 1, 10, 22) (Table 1), invoking selection against heterozygotes at multiple, presumably independent loci to explain the overall heterozygote deficiencies observed in *U. imbecillis* seems unwarranted. Certainly, this type of selection is not generally operative in *U. peggyae* or *U. peninsularis* (see Table 1). The syntopic occurrences of *U. imbecillis* with *U. peggyae* (at LRG) and *U. peninsularis* (at SR; see Appendix 1), though the disparity of the respective heterozygote deficits was maintained, do not support a hypothesis of differential underdominant selection based on habitat. Likewise, there is no reason to expect that null alleles, aneuploidy, or molecular imprinting would be more common in *U. imbecillis* and *U. "imbecillis"* than in *U. peggyae* or *U. peninsularis*.

Given the above discussion, an absolute linkage between large heterozygote deficiencies and simultaneous hermaphroditism among these species is suggestive. The most likely explanation for the relatively large heterozygote deficiencies observed in certain populations of *U. imbecillis* and *U. "imbecillis"* is a high level of inbreeding resulting from substantial self-fertilization in those particular populations. Indeed, Johnston et al. (1998) detected significant non-zero selfing rates in two of seven *U. imbecillis* populations examined individually and in the mean selfing rate for the

APPENDIX II.

Presumptive loci scored and buffer systems used in this study.

Enzyme	No. Loci Scored	Abbreviation	E.C. No.	Buffer System
Aspartate aminotransferase	1	AAT	2.6.1.1	TMME 7.4
Cytosol aminopeptidase	1	CAP	3.4.11.1	TC 8.0
Dihydrolipoamide dehydrogenase	1	DDH	1.8.1.4	MC 5.5
Esterase (alpha naphthyl acetate)	1	EST	3.1.1.-	MC 6.0
Fumarate hydratase	1	FUMH	4.2.1.2	TMME 7.4
Glucose-6-phosphate isomerase	1	GPI	5.3.1.9	MC 5.5
Glycerol-3-phosphate dehydrogenase	1	G3PDH	1.1.1.8	TC 8.0
Isocitrate dehydrogenase	1	IDH	1.1.1.42	MC 6.0 NADP
Phosphoglucumutase	1	PGM	5.4.2.2	MC 6.0
Total = 9				

References for the electrophoretic buffer systems are as follows: tris-maleic acid-magnesium chloride-EDTA pH 7.4 (TMME 7.4, Spencer *et al.*, 1964), tris-citrate pH 8.0 (TC 8.0, Selander *et al.* 1971), 3-amino propyl morpholine-citrate pH 6.0 (MC 6.0, Clayton and Tretiak 1972). The MC5.5 system was identical in composition to the MC 6.0 system except that the gel buffer was pH 5.5. When NADP was added to a particular electrophoretic system, 20 mg was added to the gel (425 ml) and 10 mg was added to the cathodal (-) electrode tray.

species; significant non-zero mean selfing rates were not detected in *U. peggyae* or *U. peninsularis*, as expected for gonochoric species. The self-fertilization hypothesis could be further tested by comparing nuclear genotypes of brooded offspring with those of the brooding parent.

Since the ratio of the volume of testicular to ovarian tissue in the gonad of a simultaneous hermaphrodite is expected to vary with mating system (Charnov 1982, Eernisse 1988), Kat's (1983) gonadal allocation data can be used to test the 'variable levels of self-fertilization' hypothesis. A positive correlation between levels of within-individual genetic variability and testicular: ovarian allocation ratios for particular populations of *Utterbackia imbecillis* would corroborate the 'variable levels of self-fertilization' hypothesis. Four populations of *U. imbecillis* were both included in this study and that of Kat (1983): BHCi, LTCi, PCi, and SRi. BHCi and PCi are from lotic habitats and LTCi and SRi are from standing water habitats (Appendix I). LTCi is allozymically monomorphic whereas SRi has a heterozygote deviation score of -1.000. BHCi and PCi have heterozygote deviation scores of -0.753 and -0.758, respectively. In Kat's (1983) study, individuals from LTCi and SRi had relatively low testicular to ovarian tissue volume ratio estimates (0.09 and 0.18, respectively); individuals from BHCi and PCi had relatively high testicular to ovarian tissue ratios (0.47 and 0.36, respectively). The evident positive correlation between the levels of within-individual genetic variability and the testicular to ovarian tissue ratio estimates (Kat 1983) for these four populations is in strong accord with predictions from sex allocation theory, i.e., self-fertilizing hermaphroditic individuals are expected to produce less spermatozoa than cross-fertilizing hermaphroditic individuals (Charnov 1982). Additional estimates of *U. imbecillis* gonadal tissue volumes and their correlation with rates of self-fertilization were presented in Johnston *et al.* (1998). Therein, individuals from high selfing rate populations had a significantly lower proportion of testicular tissue, in support of sex allocation theory.

In the absence of additional data, the stark contrast between the heterozygote deviation scores of *Utterbackia imbecillis*/*U. "imbecillis"* and *U. peggyae*/*U. peninsularis* are most readily explained by recourse to the high levels of inbreeding produced by self-fertilization. This hypothesis is supported by the (1) overall levels of within-population variation; (2) number of loci with significant departures from H-W equilibrium; (3) levels of heterozygote defi-

cit observed in the simultaneously hermaphroditic species compared with those observed in the obligately cross-fertilizing *U. peggyae* and *U. peninsularis* (Table 1); (4) the positive correlation, observed in *U. imbecillis*, between the levels of within-individual genetic variability determined herein and the testicular to ovarian tissue volume ratio estimates of Kat (1983); and (5) the positive correlation, observed in *U. imbecillis*, between the rates of cross fertilization and the proportion of male gonadal tissue (Johnston *et al.* 1998). In addition, the wide range of among-population variation in heterozygote deviation scores (see Table 1) suggests that the relative frequency of self-fertilization likely varies among populations of *U. imbecillis* and *U. "imbecillis"*.

In order to categorize populations of *U. imbecillis* s.l. by mating system type (i.e., predominantly self-fertilizing, mixed selfing and cross-fertilizing, or predominantly cross-fertilizing), criteria were used that are similar to those used by McCracken and Selander (1980) to determine the mating system assignments for terrestrial slugs. From these somewhat arbitrary criteria, 14 of the 23 (60.9%) assayed populations of *U. imbecillis* are categorized as predominantly self-fertilizing (i.e., populations having no polymorphic loci or having a heterozygote deviation score of -1.00; populations 3-6, 9, 11-14, 16, 18, 21-23; Table 1), seven (30.4%) as having a mixed mating system involving some level of cross-fertilization (i.e., populations having a heterozygote deviation score greater than -1.00 but markedly less than the lowest value seen in the obligately cross-fertilizing *U. peggyae* and *U. peninsularis* (-0.177); populations 1, 2, 8, 10, 15, 17, 20; Table 1), and two (8.7%) as having a predominantly cross-fertilizing mating system (i.e., populations having a heterozygote deviation score similar to the lowest value seen in *U. peggyae* or *U. peninsularis*; populations 7 and 19; Table 1). Similarly, three *U. "imbecillis"* populations (75%) are categorized as predominantly self-fertilizing (populations 2-4; Table 1) and one (25%) population (population 1; Table 1) is categorized as mixed mating. Natural populations of pulmonate gastropods as well as most other hermaphroditic molluscan taxa are thought to be predominantly cross-fertilizing (Foltz *et al.* 1984, Tompa *et al.* 1984, O Foighil and Eernisse 1987, Jarne *et al.* 1993, Jarne and Charlesworth 1993). Therefore, the inference that most of the *U. imbecillis* s.l. populations examined herein are undergoing some degree of self-fertilization runs contrary to expectations. No strong correlations are evident between these mat-

TABLE 1.
Within-population variation at 9 loci in *Utterbackia*.

	N	Mean # Alleles per locus & S.E.	# Polymorphic Loci (%)	# Loci with a Significant Departure from H-W Exp.	Heterozygote Deviation (D)	Mating System
Hermaphroditic Species						
<i>U. imbecillis</i> (n = 331)						
1) ACi	33	1.33 ± 0.17	3 (33)	3	-0.968	M
2) BHCi	11	1.11 ± 0.11	1 (11)	1	-0.753	M
3) CACi	4	1.00 ± 0.00	0 (0)	—	—	S
4) CCLi	7	1.00 ± 0.00	0 (0)	—	—	S
5) CDi	32	1.00 ± 0.00	0 (0)	—	—	S
6) DCLi	5	1.00 ± 0.00	0 (0)	—	—	S
7) DRLi	7	1.11 ± 0.11	1 (11)	0	-0.204	C
8) GALi	33	1.11 ± 0.11	1 (11)	1	-0.745	M
9) GLCi	14	1.00 ± 0.00	0 (0)	—	—	S
10) KLTi	18	1.33 ± 0.17	3 (33)	2	-0.825	M
11) KRK8i	7	1.00 ± 0.00	0 (0)	—	—	S
12) KRK9i	6	1.00 ± 0.00	0 (0)	—	—	S
13) KRMi	4	1.00 ± 0.00	0 (0)	—	—	S
14) LCHi	16	1.00 ± 0.00	0 (0)	—	—	S
15) LRGi	5	1.11 ± 0.11	1 (11)	0	-0.571	M
16) LTCi	12	1.00 ± 0.00	0 (0)	—	—	S
17) MCi	12	1.22 ± 0.15	2 (22)	1	-0.606	M
18) MCWi	7	1.00 ± 0.00	0 (0)	—	—	S
19) OSFi	24	1.22 ± 0.15	2 (22)	0	-0.181	C
20) PCi	12	1.11 ± 0.11	1 (11)	1	-0.758	M
21) PRWi	4	1.00 ± 0.00	0 (0)	—	—	S
22) SRi	34	1.22 ± 0.15	2 (22)	2	-1.000	S
23) UPPi	24	1.00 ± 0.00	0 (0)	—	—	S
<i>U. "imbecillis"</i> (n = 51)						
1) CRHi	11	1.11 ± 0.11	1 (11)	0	-0.632	M
2) FPCi	20	1.00 ± 0.00	0 (0)	—	—	S
3) ORTi	7	1.00 ± 0.00	0 (0)	—	—	S
4) SRGi	13	1.11 ± 0.11	1 (11)	1	-1.000	S
Mean	14.1	1.08	0.70 (7.7)	1.00	-0.687	
Gonochoic Species						
<i>U. peggyae</i> (n = 99)						
1) ACHp	16	1.56 ± 0.34	3 (33)	0	0.152	C
2) CSLp	15	1.33 ± 0.17	3 (33)	0	0.029	C
3) HCCp	8	1.44 ± 0.24	3 (33)	0	0.197	C
4) LRGP	16	1.44 ± 0.24	3 (33)	1	-0.158	C
5) LTCp	15	1.56 ± 0.34	3 (33)	0	-0.177	C
6) SHFp	19	1.22 ± 0.15	2 (22)	0	0.057	C
7) YRCp	10	1.11 ± 0.11	1 (11)	0	0.118	C
<i>U. peninsularis</i> (n = 77)						
1) GCPs	14	1.22 ± 0.15	2 (22)	0	-0.120	C
2) HRHs	13	1.22 ± 0.22	1 (11)	0	0.056	C
3) HRP	5	1.22 ± 0.15	2 (22)	0	0.364	C
4) NRBs	5	1.11 ± 0.11	1 (11)	0	0.000	C
5) RCAs	20	1.22 ± 0.15	2 (22)	0	-0.055	C
6) SRs	20	1.56 ± 0.24	4 (44)	1	-0.056	C
Mean	13.5	1.32	2.31 (25.4)	0.15	0.031	

Mating system designations are the following: C = predominantly cross-fertilizing; M = mixed (cross- and self-fertilizing); S = predominantly self-fertilizing.

ing system assignments and (1) type of aquatic habitat (lentic versus lotic systems) or (2) geographic location (cf. Table 1 and Appendix I). However, the two populations of *U. imbecillis* that have relatively small heterozygote deficiencies (DRLi and OSFi) are located at relatively low latitudes.

It should be emphasized that these mating system categories,

based on the distribution of within-population genetic variation, are rough approximations. Equating complete genetic monomorphism with a predominantly self-fertilizing mating system is especially tenuous because such homogeneity has been detected in obligately cross-fertilizing species (Bonnell and Selander 1974, Schnell and Selander 1981). However, the lack of completely

monomorphic populations in the obligately cross-fertilizing (gonochoric) species of this study is consistent with the rationale for these particular mating system assignments. The lower within-population levels of genetic variation observed in the hermaphroditic *Utterbackia imbecillis* and *U. imbecillis*, relative to congeneric gonochores, and the extremely high mean F_{ST} value for the well-sampled *U. imbecillis* suggest that mating system differences have had an impact on the intraspecific distribution of genetic variation within *Utterbackia*. Significant levels of selfing, in combination with genetic drift, have likely reduced the within-population component of genetic variation in the hermaphroditic species of *Utterbackia* while augmenting genetic differentiation among populations of *U. imbecillis* through founder effects.

Inferences Regarding the Dispersal Capability of Utterbackia imbecillis

Species in the predominantly gonochoric Unionidae typically exhibit a relatively high degree of drainage-basin philopatry (LaRocque 1967, Burch 1975a, McMahon 1991). For example, no unionid species is native to both sides of the North American continental divide. Additionally, very few species have ranges that include portions of both the Mississippi River and Atlantic Slope drainages. In these instances, it is apparent that mountain ranges are acting as formidable barriers to dispersal for unionid bivalves. Even in cases where there are no obvious barriers to dispersal (e.g., the low coastal plain habitat that is currently separating *U. peggyae* and *U. peninsularis*) or obvious habitat differences, unionid species' distributional patterns suggest that inter-drainage colonization is a relatively rare event.

In contrast, the distributional ranges exhibited by the simultaneous hermaphroditic, freshwater bivalves of the family Sphaeriidae are relatively cosmopolitan in nature (LaRocque 1967, Burch 1975b). Many native North American sphaeriids have transcontinental distributions (McMahon 1991, Burch 1975b). Self-fertilization is apparently common in sphaeriid bivalves (Thomas 1959, Mackie 1984) and the ability of single, self-fertilizing sphaeriid individuals to colonize new habitats is postulated as one of the major factors responsible for the relatively cosmopolitan species distributions in this taxon (McMahon 1991). Therefore, the self-fertilization potential of *Utterbackia imbecillis* may largely account for its atypically (i.e., for a gonochoric unionid bivalve) widespread geographic distribution in eastern North America. Additional evidence consistent with relatively high dispersal capability for *U. imbecillis* includes the (1) postulated southeastern USA origin for this now-widespread species (Hoeh et al. 1995), (2) lack of among-population resolution for *U. imbecillis* in a phylogenetic analysis of *Utterbackia* (Hoeh et al. 1995) and (3) reports of recent range expansion into areas where it has been historically absent (Starrett 1971, Fuller and Hartenstine 1980, Kat 1983, Hoeh et al. 1995).

Further investigation is necessary to understand a somewhat paradoxical finding of this study with regard to *U. "imbecillis"*. An initial hypothesis postulated that this geographically restricted, simultaneous hermaphrodite was predominantly cross-fertilizing (Hoeh et al. 1995). However, analyses of within-population genetic variability (Table 1) suggest that *U. "imbecillis"* populations, like many of those of *U. imbecillis*, are not predominantly cross-fertilizing. If self-fertilization typically facilitates dispersal in freshwater bivalves, what factor(s) is limiting the geographic range of *U. "imbecillis"*?

The range of *U. "imbecillis"* is in an area of the southern Atlantic Slope that has a relatively large number of ichthyofaunal endemics (Lee et al. 1980, Shute et al. 1981, Wiley and Mayden 1985, Hocutt et al. 1986, Swift et al. 1986, Wood and Mayden 1992). If *U. "imbecillis"* has an obligate host-fish association with one or more of the endemic fish species of the region, this would preclude its movement into other drainages lacking the required fish hosts. A determination of the host-fish requirements of *U. "imbecillis"* is necessary to test this hypothesis. However, the dependence of *U. "imbecillis"* on other unique attributes of the drainages of the southern Atlantic Slope cannot be ruled out at the present time. A relatively recent transition to predominantly self-fertilization is another possibility that would explain the relatively small geographic range of *U. "imbecillis"*.

Evolution of Mating Systems in Utterbackia imbecillis

Of broad interest (Ghiselin 1969, 1974, Maynard Smith 1978, Charnov 1982, Lloyd 1988, Jarne and Charlesworth 1993) is the elucidation of the processes involved in the maintenance of the wide range of mating systems (predominantly cross-fertilizing through predominantly self-fertilizing) inferred for *Utterbackia imbecillis* s.l. in this report. This range of mating systems may represent multiple optimal end points generated by local selective regimes or, in the case of the mixed-mating system populations, merely transient states on the way toward either predominantly cross- or self-fertilization. However, the lack of (1) long-term studies of the interpopulational variation in mating systems, (2) knowledge of ecological requirements, and (3) knowledge of fertilization biology in *U. imbecillis* currently precludes a deeper understanding of the evolutionary forces shaping the mating system in this species.

The fertilization biology of the gonochoric, let alone the simultaneously hermaphroditic, unionid bivalves is, at best, poorly understood. Fertilization in unionids is thought to take place within the female suprabranchial chambers or in the marsupia (gills modified for larval brooding) in gonochoric species, but the details of the process are unknown (Coker et al. 1921, Matteson 1948). However, it appears that in multiple species of gonochoric unionids, representing different subfamilies, spherical spermatozoa aggregates are released by males prior to fertilization (Utterback 1931, Edgar 1965, Lynn 1994). That these aggregates are released from male unionids from diverse taxa suggests that this conserved feature may be essential for cross-fertilization. Data from *Pyganodon grandis* (Unionidae: Anodontinae) suggest that females collect these spermatozoa aggregates with their gills and store the spermatozoa in their nephridia (W. R. Hoeh and R. J. Trdan unpubl. data). Subsequently, the spermatozoa are likely released from the nephridial pore as oocytes are released from the adjacent genital opening, thus effecting fertilization.

In simultaneously hermaphroditic unionids, a common gonoduct carries both oocytes and autospermatozoa to the suprabranchial chambers (Mackie 1984). This suggests that the oocytes of a simultaneous hermaphrodite may encounter autospermatozoa in the common gonoduct before encountering allospermatozoa (which may be retained in the suprabranchial chambers or brood pouches?). The use of a common gonoduct may favor self-fertilization unless the release of male and female gametes can be displaced in time. Observations made during the course of a preliminary examination of the reproductive biology of *Utterbackia imbecillis* (Hoeh et al. 1986) noted a concurrent release of oocytes

and, presumably, autospermatozoa in the gonoducts of individuals from four of the populations examined in the present study (ACi, BHCi, CDi, MCi). Furthermore, the spermatozoa within the gonoducts were not contained in spherical aggregates as mentioned above for male unionids. The individuals examined were from populations that were likely undergoing significant amounts of self-fertilization (see discussion above and Table 1).

These limited observations on the fertilization biology of *U. imbecillis* suggest that (1) the use of a common gonoduct in simultaneous hermaphrodites may predispose the mating system towards self-fertilization and (2) all spermatozoa may not be capable of effecting cross-fertilization. More information regarding the details of fertilization biology for both gonochoric and hermaphroditic unionids is required to address these possibilities. Specifically, comparative information on the fertilization biology of individuals of *U. imbecillis* from populations that were categorized, herein, as self-fertilizing, mixed mating, and cross-fertilizing would be most illuminating. Questions still to be addressed include (1) Does the selfing rate vary among individuals within *U. imbecillis* s.l. populations? (2) Is the selfing rate within a *U. imbecillis* s.l. population relatively fixed or plastic over time? (3) Is the selfing rate within populations of *U. imbecillis* s.l. correlated with particular environmental variables? The answers to these questions will be of broad interest and utility to evolutionary, conservation, and mollusk biologists.

Conservation Implications

Bivalves in the family Unionidae are one of the most endangered groups of North American aquatic macroinvertebrates (Bogan 1993, Williams et al. 1993, Lydeard and Mayden 1995). However, unionid conservation and management plans have been hampered by a relative lack of knowledge concerning (1) unionid ecological requirements (e.g., fish host and dietary), (2) the actual extent of unionid biodiversity, (3) the distribution of genetic variation within species, and (4) mating systems. Studies such as this one are useful for assessing the distribution of genetic variation within species and to evaluate the effects of mating system variation on the distribution of genetic variation (Hamrick and Godt 1990). Knowledge of a species' genetic structure can provide important insights into population-level processes such as colonization, extinction, and gene flow as well as for conservation/management initiatives (Slatkin 1977, 1985, 1993, Falk and Holsinger 1991, McCauley 1991, 1992).

The superior colonization potential of self-fertilizing species, such as *Utterbackia imbecillis*, may be partially offset, in an evolutionary sense, by the reduction of within-population genetic variation due to the combined effects of selfing and founder events. For instance, the relatively large geographic range of *U. imbecillis* will likely act to buffer this species from complete extinction. However, the relatively low levels of within-population genetic variation observed in *U. imbecillis* s.l., with respect to *U. peggyae* and *U. peninsularis*, may predispose individual populations of these hermaphroditic species to increased probability of extinction due to reduced ability to cope with both natural (Thorpe et al. 1981, Allendorf and Leary 1986, Lively et al. 1990) and anthropogenic (Guttman 1994) environmental change. Therefore, extinction and colonization are potentially important population-level processes within *Utterbackia*, and metapopulation-dynamic models may be especially applicable to the hermaphroditic *Utterbackia* species (Hastings and Harrison 1994).

The relatively high F_{ST} values reported herein for four species of *Utterbackia* indicate that much of the genetic variation within each species is distributed among populations, suggesting relatively low levels of historical gene flow. An especially high F_{ST} value (0.818) was obtained for *U. imbecillis*. This latter result, which is counter intuitive for an able colonizer such as *U. imbecillis*, is likely an outcome of significant levels of self-fertilization, which leads to a within-individual reduction of genetic variability, combined with frequent population extinctions and single-individual founder events facilitating the fixation of distinct alleles in different populations (Wade and McCauley 1988, McCauley 1991, McCauley 1992). Therefore, the normally homogenizing effects of dispersal are likely potentiating among-population differentiation within *U. imbecillis*.

Overall, the relatively high F_{ST} values observed herein within four species of *Utterbackia* have significant implications for potential conservation plans for these species. Since the among-population component of genetic variation is relatively high, any conservation initiative to preserve a significant amount of the genetic diversity within species of *Utterbackia* would necessitate the protection of multiple populations of each species. One potential strategy, consistent with the findings of this report, would be to protect one or more populations within each distinct drainage basin for each *Utterbackia* species. Additionally, transplantation initiatives, to aid population recovery efforts, should take into consideration the genetic structure of the target species. Transplantations of individuals between genetically distinct populations could lead to (1) the detriment of the recipient population due to outbreeding depression if cross-fertilization occurs (Templeton 1986), (2) the introduction of genotypes that are poorly adapted to the new ecological conditions (Huenneke 1991), and (3) loss of the original genotypes due to the potential superior competitive ability of the transplanted individuals' genotypes. Clearly, the preservation of a species' overall genetic diversity is desirable for its long-term survival and is dependent upon an understanding of the genetic structure of the constituent populations (Hamrick et al. 1991, Saccheri et al. 1998).

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APPLICATION OF MOLECULAR GENETIC MARKERS TO CONSERVATION OF FRESHWATER BIVALVES

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ABSTRACT Freshwater bivalves (Unionacea) are among the most endangered faunal elements in North America. Molecular genetic studies have much to offer conservation efforts directed to this declining fauna. Molecular genetic data can provide information needed to identify evolutionarily significant units, resolve taxonomic ambiguities, describe population structure, evaluate impacts of habitat fragmentation and reduced gene flow among populations, reconstruct phylogenetic relationships, clarify fish host-glochidia relationships, and provide evidence in legal actions. Molecular genetic techniques and their application to freshwater bivalves are reviewed.

KEY WORDS: Freshwater bivalves, Unionacea, genetics, conservation, molecular markers

INTRODUCTION

Freshwater bivalves are among the most endangered of animal groups. Reductions in the number of species and in the abundance of freshwater bivalve populations have been reported worldwide (Bogan 1993, Williams et al. 1993, Ziuganov et al. 1994, Lydeard and Mayden 1995, Williams and Neves 1995, Abramovitz 1996). Dam construction, channelization, pollution, commercial exploitation, and introduction of exotic species contribute to extinction, population fragmentation, and reduction in population sizes. Changes in dispersal and gene flow between populations and reduced effective population size occur concomitantly with these environmental perturbations and may lead to loss of genetic diversity. Therefore, in addition to concerns about the outright loss of freshwater bivalves, conservation strategies must consider the potential ecological and evolutionary impact of changes in genetic characteristics.

Genetic diversity has been shown to be relevant to population health and probability of persistence (O'Brien and Evermann 1988, Quattro and Vrijenhoek 1989). Numerous studies of marine bivalves report a positive relationship between genetic diversity and surrogate measures of fitness such as growth (Garton et al. 1984, Koehn et al. 1988), fecundity (Rodhouse et al. 1986), and survival (Diehl and Koehn 1985). Although this relationship has not been extensively studied in freshwater bivalves, a comparable relationship may be expected. Genetic diversity should be conserved because of its immediate contribution to fitness-related traits, and because genetic diversity is an essential component of adaptation and evolutionary success.

Molecular genetic markers provide powerful tools to investigate ecology, demography, biogeography, and evolutionary history. Genetic markers can be used to determine if a group of organisms constitutes a species, subspecies, or population. Determination of species status has direct and immediate application to conservation decisions as well as being of general interest to evolutionary biology. More specifically, conservation biologists interested in freshwater bivalves are concerned with several issues that

can be approached from a molecular genetic perspective. These issues include identification of Evolutionarily Significant Units (ESUs) and Management Units (MUs), systematics and taxonomy, spatial patterns of variation (intraspecific phylogeny), gene flow, hybridization, inbreeding, bivalve-fish host relationships, and forensics. Currently, there are few molecular genetic studies of freshwater bivalves.

Several recent publications provide excellent summaries of molecular genetics and applications to conservation issues (Avice 1994, Moritz 1994a, O'Brien 1994, Avice and Hamrick 1996, Hillis et al. 1996, Ferraris and Palumbi 1996, Haig 1998). Here, we provide only a brief technical review and summarize information regarding molecular genetic markers and freshwater bivalves. Our focus is on conservation issues of freshwater bivalves and molecular approaches that may help address these issues.

MOLECULAR GENETIC MARKERS

Morphological and physiological phenotypes are often under complex polygenic control, subject to environmental perturbations, and may be direct targets for selection. In contrast, molecular genetic markers (protein and DNA) have simple genetic underpinnings and most can be considered to behave as neutral markers. Information contained in these molecules can be used to evaluate population and evolutionary processes. Selection of appropriate molecular genetic markers, from the many that are available, is critical. Of primary importance, the marker must provide genetic variation appropriate to the question. Secondary considerations include time required to process samples, specialized equipment or training, and cost (Table 1).

Protein Electrophoresis

Protein electrophoresis provides a convenient, reliable, and cost-effective tool to study population genetic processes. A general overview of electrophoretic procedures and specific buffers and staining methods can be found in Richardson et al. (1986) and Hillis et al. (1996). Proteins are separated on or in a supporting

TABLE 1.

Comparison of methods available for molecular genetic studies and their routine application and relative cost.

Methods	Application ^a	Genome	Number of Loci	No. Individuals	Cost
Protein electrophoresis	CP, CRS	Nuclear	Many	Many	\$
Restriction fragment length	CP, CRS	mtDNA,	Few	Many	\$\$
Polymorphism (RFLP)		Nuclear			
Random amplified	CP, CRS	Nuclear	Many	Many	\$\$
Polymorphic DNA (RAPD)					
Microsatellites	CP	Nuclear	Few to many	Few to many	\$\$
DNA Sequencing	CP, CRS, DRS, DP	mt DNA	Few	Few	\$\$\$
		Nuclear			

CP—conspecific populations, CRS—closely-related species, DRS—distantly related species, DP—deep phylogenetic reconstruction.

medium (e.g., starch, polyacrylamide, and cellulose acetate) by charge differences associated with changes in amino acid sequence and/or by size. Specific enzymes or proteins are visualized using histochemical stains. The underlying genetic basis for most banding patterns is well established and interpretation of banding patterns follows rules of simple Mendelian inheritance. One or more loci may be visualized and each locus may have one or more alleles. Thus, protein electrophoresis can provide information for numerous independent loci throughout the genome for genetic study. The following information is routinely obtained with allozyme studies: number of alleles per locus, percent polymorphic loci, heterozygosity, tests for fit of data to random mating expectations, estimates of population differentiation (F-statistics, contingency tables), and genetic distance and identity measures. Numerous programs are available for analysis of electrophoretic data including BIOSYS-1 (Swofford and Selander 1981) and others described in Hillis et al. (1996).

DNA Approaches

The application of DNA-based molecular genetic markers involves a consideration of nuclear versus mitochondrial genomes. The mitochondrial genome of bivalves, like that of other animals, is about 18 kilobase (kb) pairs in length and composed of about 37 genes. These genes code for tRNAs, rRNAs, and proteins involved in electron transport and oxidative phosphorylation. The mitochondrial genome represents a single, complex linkage group. Although mtDNA is inherited through maternal lines in many animals, in bivalves gender-specific genomes have been reported and the pattern of inheritance is described as doubly uniparental inheritance (DUI) (Skibinski et al. 1994, Zouros et al. 1992, 1994). Male and female mtDNA genomes are nonrecombining and highly divergent (Hoeh et al. 1996, Liu et al. 1996b). Regions of the mtDNA differ in their rates of divergence and in their utility for studies at different hierarchical levels of analysis from populations to systematics.

The nuclear genome is not as well studied as the mitochondrial genome. Single-copy gene sequences can be obtained via the polymerase chain reaction (PCR) using specially designed primer pairs (e.g., Karl and Avise 1993). Nuclear rRNA genes consist of tandemly repeated units (Fig. 1a) and have been examined in freshwater bivalves (Liu and Mulvey, unpubl). The repeated units may occur in high copy numbers and may be dispersed throughout the genome. Despite the large copy number there is a high degree of homogeneity in these regions (Hamby and Zimmer 1992). In a

study of freshwater bivalves in the genus *Elliptio*, Liu and Mulvey (unpubl) found that the internal transcribed spacer rejoins (ITS1 and ITS2) were highly variable whereas the rRNA coding regions (18S, 5.8S, and 28S) were conserved. Segments of the rRNA repeat unit may be useful in studies of freshwater bivalves including populations studies (spacers) and phylogenetic reconstruction (rRNAs).

DNA Extraction

Foot, mantle, gill, gonad, and muscle tissue have been used to obtain DNA for analysis. Fresh, frozen, and ethanol-preserved specimens are good sources for DNA samples. Nondestructive sampling methods have been described for bivalves (Stiven and Alderman 1992, Berg et al. 1995). These methods will be especially useful when endangered or threatened species are studied. DNA extraction from freshwater bivalve tissues can be accomplished using a variety of methods including standard phenol-chloroform (Sambrook et al. 1989), and chelex (Walsh et al. 1991). Methods have recently been described for use of formalin-fixed specimens for DNA isolation and PCR amplification (Shedlock et al. 1997). Thus, historical museum collections of freshwater mussels may now be accessible to modern molecular genetic analysis.

Polymerase Chain Reaction

The PCR takes advantage of thermo-stable DNA polymerases to amplify DNA sequences from template DNA to provide large quantities of specific sequences. PCR requires only a small amount of template DNA; thus, this approach is well suited to endangered species and conservation. PCR products can then be used in subsequent analyses as described below. PCR requires primers, which are short DNA fragments, to initiate DNA synthesis. Primers may be random or gene-specific. Primer development was a limitation in the application of this technique early in its development but primer design has been greatly facilitated by the availability of sequence data (Genbank, EMBL, and other resources).

Numerous primers have been successfully applied in studies of freshwater bivalves (Table 2). As illustrated in Figures 1a, b, and c, primer pairs can be selected to obtain PCR products for several regions, to select the size of the fragment, and to select for regions that are "fast-evolving" or "slow-evolving." Additionally, many of the "universal" or kit primers may be useful in studies of freshwater bivalves but have not yet been widely applied.

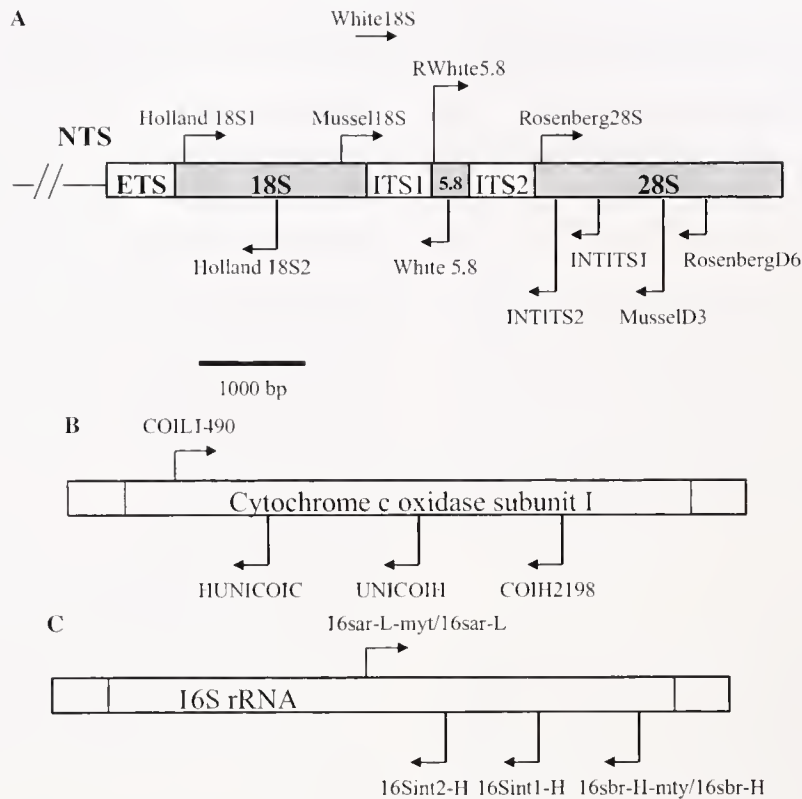


Figure 1. Location of primers used for PCR amplification of DNA for freshwater bivalves. (a) rRNA array, (b) COI, (c) 16S rRNA. Primers are listed in Table 2.

DNA Markers

Restriction Fragment Length Polymorphisms

Restriction fragment length polymorphisms (RFLP) are obtained when restriction enzymes cleave double-stranded DNA.

These enzymes have specific recognition and cleavage sites usually of four, five, or six base pairs. Data are generated by digestion of DNA with a series of restriction enzymes and size-based separation of the resulting fragments using gel electrophoresis (agarose or acrylamide). The number and size of the resulting fragments

TABLE 2.
Primers that have been used in DNA sequencing studies of freshwater bivalves.

Gene Segment	Primer Label	Primer Sequence	Reference
COI	UNICOIH	5'-TCA GCA ACC AAC CCA GGA G-3'	Roe & Lydeard (unpubl)
	HUNICOIC	5'-AAC AAC ACT CTC TAC CAA AG-3'	Roe & Lydeard (unpubl)
	COIL 1490	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'	Folmer et al. 1994
	COIH 2198	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Folmer et al. 1994
16S rRNA	16sar-L	5'-CGC CTG TTT ATC AAA AAC AT-3'	Palumbi et al. 1991
	16sbr-H	5'-CCG GTC TGA ACT CAG ATC ACG T-3'	Palumbi et al. 1991
	16sar-L-myt	5'-CGA CTG TTT AAC AAA AAC AT-3'	Lydeard et al. 1996
	16sbr-H-myt	5'-CCG TTC TGA ACT CAG CTC ATG T-3'	Lydeard et al. 1996
	16Sint1-H	5'-GAAA ARG TAA AGY TCC GC-3'	Lydeard et al. 1996
	16Sint2-H	5'-RGR TTG CCC CAA TCH HHC-3'	Lydeard et al. 1996
	16Sint2-H	5'-RGR TTG CCC CAA TCH HHC-3'	Lydeard et al. 1996
18S rRNA	Holland 18S1	5'-GCC AGT AGC ATA TGC TTG TCT C-3'	Adamkewicz et al. 1997
	Holland 18S2	5'-AGA CTT GCC TCC AAT GGA TCC-3'	Adamkewicz et al. 1997
ITS1	Mussel18S	5'-TCC CTG CCC TTT GTA CAC ACC G-3'	Liu & Mulvey (unpubl)
	WHITE18S	5'-TAA CAA GGT TTC CGT AGG TG-3'	White et al. 1994
	WHITE5.8	5'-AGC TRG CTG CGT TCT TCA TCG A-3'	White et al. 1994
ITS2	RWHITE5.8	5'-TCG ATG AAG AAC GCA GCY AGC T-3'	White et al. 1994
	INTITS2	5'-TTT TCC CTC TTC ACT CGC CGT TAC-3'	Liu & Mulvey (unpubl)
28S rRNA	Rosenberg28S	5'-GCG GAG GAA AAG AAA-3'	Rosenberg et al. 1994
	INTITS1	5'-CGT GGC AAT CAA CCC GAG GAA AGT-3'	Liu & Mulvey (unpubl)
	MusselD3	5'-CCT TCT CAG GCA TAG TTC ACC ATC-3'	Liu & Mulvey (unpubl)
	RosenbergD6	5'-CTA CTA CCA CCA AGA TCT GC-3'	Rosenberg et al. 1994

depend on the number and distribution of recognition sites. Variation in fragment patterns arises from base pair substitutions, insertions or deletions, sequence rearrangements, or differences in the overall size of the target DNA. Data consist of restriction fragment lengths, which are scored as present or absent or restriction sites which are map locations. In the latter case, scores consist of the presence or absence of recognition sequences. Software available for the analysis of RFLP data includes RESTSITE (Miller 1991) and RESTML (Felsenstein 1993).

Many RFLP studies involve digestion of the intact mitochondrial genome. Mitochondrial DNA is obtained following isolation and purification of mitochondria using cesium-chloride gradient centrifugation prior to digestion with restriction enzymes. The development of PCR and associated techniques has made it possible to apply the RFLP method to PCR products from nuclear and mitochondrial genomes. A general scheme for RFLP data generated for the ITS1 region of *Elliptio* is shown in Figure 2. RFLP data are used to evaluate population differentiation and to reconstruct phylogenies.

Random Amplification of Polymorphic DNA

Random amplification of polymorphic DNA (RAPD) uses short (approximately 10 bp) primers to amplify random, anonymous sequences with PCR (Williams et al. 1990). Thus, no *a priori* knowledge of sequences is needed. A single primer is used and PCR products are fragments flanked by sequences complementary to the primer. Data consist of presence or absence scores for size-separated fragments on polyacrylamide or agarose gels. Polymorphisms display dominant-recessive patterns. Numerous primers are commercially available to facilitate screening for informative markers. This method is particularly useful, when crosses are done to verify inheritance patterns. RAPD markers are usually applied

to intraspecific analysis. Software available for the analysis includes RAPDistance (Felsenstein 1993).

Microsatellite DNA

Microsatellite loci are a class of highly polymorphic markers identified by tandem repeats of short (2–4 bp) DNA sequences (e.g., AC_n or CTG_n , where n = number of tandem repeats). Variation arises primarily from changes in copy number of the repeated motif. These behave as simple co-dominant Mendelian polymorphisms and are readily scored when microsatellite fragments generated via PCR are size-separated on nondenaturing 6% polyacrylamide or 3–5% agarose gels. Multiple loci may be analyzed simultaneously (multiplexed) with a single PCR reaction when fragment sizes are sufficiently different to allow identification. Microsatellites are especially well suited to study genetic variation within and among conspecific populations. A limitation to widespread application of the microsatellite technique is the difficulty in identifying loci and generating appropriate primer pairs for PCR amplification. Additionally, primers are usually species-specific so they must be developed for each application. Microsatellite analyses provide multilocus genotypes that can be analyzed in a manner similar to that available for protein electrophoresis. Software for analysis includes Misat (Nielsen 1997), and MicroSat (available via anonymous ftp at lotka.stanford.edu/microsat.html).

DNA Sequencing

DNA sequence data provide the most direct assessment of genetic characteristics. Methods discussed above are indirect (protein electrophoresis) or incomplete (RFLP) approaches to genomic sequences. DNA sequence data are becoming widely used because of the availability of PCR techniques and automated DNA se-

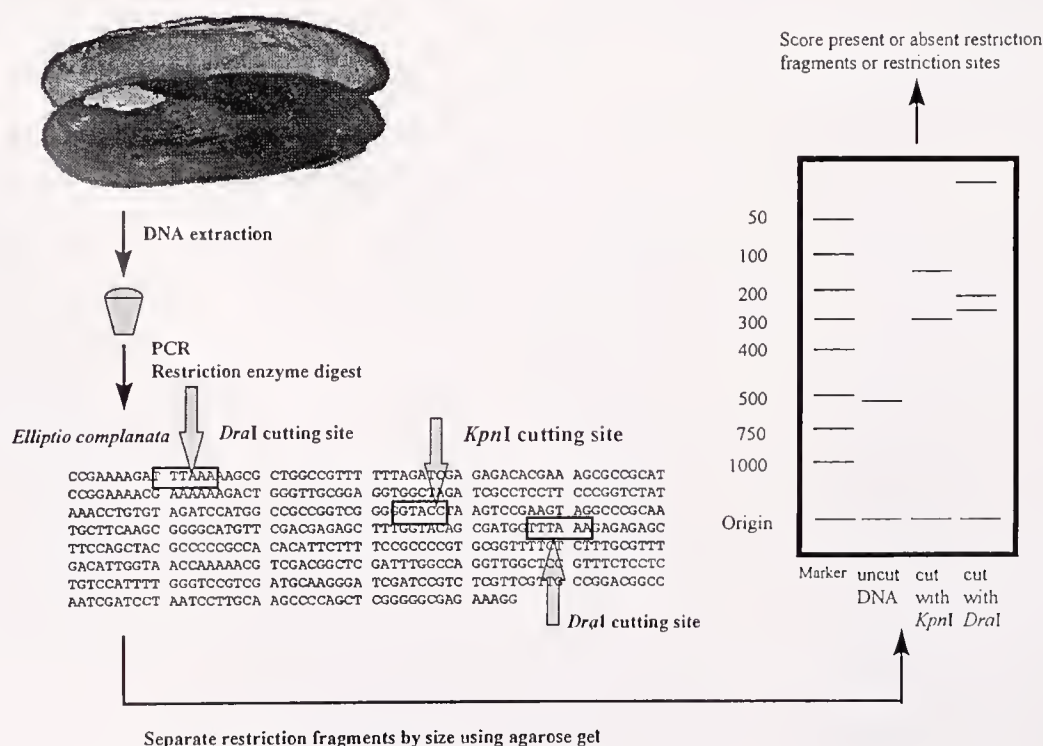


Figure 2. Overview of RFLP analysis of the ITS1 region of *Elliptio* cut with restriction enzymes *KpnI* and *DraI*.

quencing methods. Unlike the short primers used for RAPD analysis, primers used to generate sequence data are usually 20–30 bp. This length ensures specificity to the target DNA segment in the PCR. Sequencing reactions usually involve chain termination with ddNTPs to generate fragments with different lengths at termination. Fragments are separated in polyacrylamide gels and read from autoradiograms or with automated scanning devices.

One potential difficulty with DNA sequence analysis is sequence alignment. Sequence data from different samples must be homologous to allow appropriate comparisons to be made. Alignment is usually not too problematic for protein-coding genes but becomes increasingly difficult for rRNA (especially loop segments) and noncoding regions. Sequence alignment can be facilitated with programs such as Sequencher™ and ClustalV as described in Hillis et al. (1996). Loop or noncoding regions can be aligned with the help of secondary structure programs such as RNAviz (De Rijk and De Wachter 1997) and DCSE (De Rijk and De Wachter 1993). Alignments should be verified by visual inspection. Following successful alignment of homologous sequences, data can be analyzed using PAUP*4.0 (Swofford 1998), PHYLIP (Felsenstein 1993), MEGA (Kumar et al. 1993), and Hennig86 (contact biodl@wuvn.gwu.edu).

CONSERVATION APPLICATIONS FOR MOLECULAR GENETIC DATA

Application of molecular genetics to conservation of freshwater bivalves has great potential that has only just begun to be realized. Below we review available literature regarding the application of molecular genetics to freshwater bivalves and speculate on some uses for the future.

Evolutionarily Significant Unit

The concept of the evolutionarily significant unit (ESU) was advanced by Waples (1991) and later Moritz (1994b) to address the issue of genetic distinctiveness as recognized by the Endangered Species Act (ESA). The ESU concept integrates genetic, phenotypic, life history, ecological, and geographic information to identify units that are independent over evolutionary time. With the recognition of ESUs, conservation efforts are directed to the evolutionary legacy of the species. ESUs reflect long periods of genetic isolation and deep phylogenetic subdivisions. Management units (MU) represent more recent separations and shallower genetic subdivisions. Bowen 1998, and Roe and Lydeard (1998) provide extensive comment on the ESU concept and its application to molluscan conservation.

The ESU concept has rarely been explicitly applied to bivalves; however, as the following examples illustrate, this concept may have broad utility for conservation of freshwater bivalves. Roe and Lydeard (unpubl) argue for the recognition of ESUs in *Potamilus alatus* based on genetic data. Because genetic differentiation (based on DNA sequences) between the two extant populations from the Amite River and Black Warrior River was significant, Roe and Lydeard argue that they represent separate targets for conservation measures. Additionally, they suggested that specific designation be given to the Amite River form. Similarly, Liu et al. (1996a) recommended that *Pyganodon grandis* from the Arkansas River and South Platte River drainages be managed as separate units (MUs). Populations in these drainages exhibited significant genetic differentiation, especially for the male type mitochondrial genome. King et al. (1997) used an RFLP approach with the ITS1

and COI genes to assess genetic differentiation in populations of the green floater, *Lasmigona subviridis*. They found significant differences between a Pennsylvania population and populations from West Virginia and North Carolina. King et al. (1997) argue for a conservative approach, based on an assumption of genetic distinction, in the management and conservation of freshwater bivalves.

Taxonomy

Recognition of an entity as an ESU or as a distinct species is controversial, and no simple criteria for the distinction are available. The complexity and duration of the life history of freshwater bivalves preclude the use of reproductive compatibility as a criterion for species delineation; therefore, there has been strong reliance on conchological characters. However, phenotypic plasticity and convergence of form have led to confusion in many taxonomic groups. Early malacologists clearly overestimated the number of taxa (Boss 1971); however, there is growing evidence that subsequent synonymizing has oversimplified the situation (e.g., *Elliptio*, Davis and Mulvey 1993).

Taxonomy forms the basis for legal protection under the ESA. Taxonomic uncertainty presents a problem for the conservation biologist because, although taxa may be endangered or threatened, without valid taxonomic names they cannot be evaluated for protection under the ESA. Additionally, phylogenetic distinction is often considered in species recovery plans and resource allocation. As Daugherty et al. (1990) stated, "good taxonomies are not irrelevant abstractions, but the essential foundations of conservation practice."

Molecular genetic approaches are often useful to clarify relationships. Ambiguous taxonomic placements often occur, where morphological criteria used to distinguish taxa exhibit phenotypic plasticity. For such approaches to be useful, it is necessary to estimate genetic differentiation within and between species. However, many endangered species are already too rare to permit sufficient sampling for such studies, and attempts to reconstruct historical demography are often complicated by the absence of independent estimates of variation prior to exploitation or bottlenecks. Additionally, it is difficult to draw conclusions when populations are allopatric. Comparative studies provide evidence for generalities regarding population genetic patterns and processes. A comparison of genetic diversity between common and rare species can provide useful guidelines for managers. Although genetic distance values alone cannot be used to establish taxonomic distinctions, these values can be compared with others to evaluate ranges of differentiation for populations, species, genera, etc. (see Table 3 for examples of allozyme data). Hoeh and Gordon (1996) provide cautionary comments on applications and implications of molecular data for taxonomic issues. Davis (1983) examined bivalves in the genera *Unio* and *Elliptio* using allozyme electrophoresis and morphological characteristics. These genera are very similar conchologically. In addition to clearly distinguishing the two genera, the allozyme data revealed three cryptic species among individuals previously recognized as a single species, *U. tetralasmus* (Davis 1983). In this case, convergent morphologies obscured underlying genetic distinctions.

In contrast, a recent study of molecular genetic characteristics in the genus *Pleurobema* suggests that forms named on the basis of conchological features may not exhibit significant genetic differentiation (Kandl et al., unpubl). *Pleurobema reclusum* is described

TABLE 3.
Summary of genetic characteristics of freshwater bivalves determined from allozymes.

Taxon	No. Pop. or Species	No. Loci	P	H	Genetic Distance	Reference
Populations within species						
<i>Anodonta cataracta</i>	5				0.034 ± 0.038	Davis 1994
<i>Elliptio complanata</i>	11	8	0.357–0.500	0.041–0.084	0.065 ± 0.039	Davis et al. 1981 (cited in Davis 1994)
<i>Lampsilis cariosa</i>	3	11	0.636–0.818	0.260–0.318	0.071 ± 0.027	Stiven & Alderman 1992
<i>Lampsilis radiata</i>	5	7	0.071–0.357	0.004–0.041	0.018 ± 0.010	Kat & Davis 1984
<i>Leptodea ochracea</i>	2	11	0.273–0.364	0.051–0.100	0.018	Stiven & Alderman 1992
<i>Pleurobema pyriforme</i>	9	13	0.000–0.231	0.000–0.154	0.031 ± 0.029	Kandl et al. 1997
Species within genus						
<i>Amblema</i>	3	14	0.917		0.219 ± 0.025	Mulvey et al. 1997
<i>Anadonta</i>	3	14	0.113–0.357	0.028–0.107	0.457 ± 0.073	Kat 1983a (cited in Davis 1984, 1994)
<i>Elliptio</i>	7	14	0.280–0.470	0.094–0.146	0.210 ± 0.017	Davis 1981 (cited in Davis 1984, 1994)
<i>Lampsilis</i>	6	14	0.262–0.600	0.038–0.113	0.609 ± 0.478	Kat 1983b (cited in Davis 1984, 1994)
<i>Megalanaia</i>	2	14	0.617		All < 0.100	Mulvey 1997
<i>Pleurobema</i>	2	13	0.000–0.308	0.000–0.154	0.185 ± 0.045	Kandl et al.
<i>Unio</i>	3	14	0.290	0.107	0.308 ± 0.165	Davis 1981 (cited in Davis 1984, 1994)

from the Ochlockonee and Suwannee rivers of Florida. *P. pyriforme* from the Apalachicola River system to the Suwannee River system of Florida and Georgia, and *P. bulbosum* from the Chipola River of Florida. Clench and Turner (1956) suggested that these taxa, *P. pyriforme*, *P. reclusum*, and *P. bulbosum*, represented a single polytypic form, *P. pyriforme*. The distinctiveness of *P. reclusum*, *P. bulbosum*, and *P. pyriforme* has been the subject of several works (Johnson 1970, Burch 1975, Heard 1979). Kandl et al. (unpubl) used allozyme, RFLP, and DNA sequence data (COI) to determine whether specimens from the eastern Gulf drainages exhibited genetic differentiation consistent with taxonomic designations or with the hypothesis of a single polytypic species. Little or no genetic differentiation was observed among forms recognized as *P. reclusum*, *P. bulbosum*, and *P. pyriforme* although significant genetic differentiation was found between these and *P. strodeanum* from the Escambia and Yellow rivers of Florida and Alabama. Kandl et al. suggest that *P. pyriforme* is a widely distributed, conchologically variable species and that includes *P. reclusum* and *P. bulbosum*. In 1998, "endangered" status was proposed for *P. pyriforme* (U.S. Fish and Wildlife Service 1998). The taxonomic revision suggested by the work of Kandl et al. would not alter the conservation status of *P. pyriforme*.

Population Structure—Intraspecific Phylogeography

Freshwater environments are spatially and temporally heterogeneous and genetic differentiation may reflect differences associated with local environments. River systems can be considered habitat "islands" on the larger continental landscape; each river occupies a distinct basin and is separated from other rivers by habitat unsuitable for bivalves. Riverine systems exhibit varying degrees of contemporary and historical connectedness and therefore opportunities for gene flow. Northern glaciated rivers may have been colonized as recently as 10,000 years ago. Southern rivers were unglaciated and generally support more total species and more endemic species than northern rivers. In addition to natural barriers, man-made impediments (e.g., dams and channelization) to gene flow occur on most major river systems in the U.S.

Habitat fragmentation and thus fragmentation of gene pools is common for freshwater species. The distribution of genetic vari-

ability among fragmented populations depends on the distribution of genetic variability before fragmentation, the size and number of fragments, the duration of fragment isolation, mating systems, and dispersal capabilities. Population fragmentation is likely associated with loss or redistribution of genetic variability and is a concern for conservation biologists attempting to manage the genetic legacy of freshwater bivalve species.

Molecular genetic methods provide an assessment of the amount and partitioning of genetic diversity necessary to evaluate the impacts of the current extinction crisis in freshwater mollusks. For many bivalve taxa, large-scale genetic assessments are no longer possible because there are practical and legal limitations on direct assessment of genetic patterns in rare or endangered species. Determination of genetic characteristics in related taxa may provide surrogate data to evaluate endangered populations and species. Freshwater bivalves display a wide range of genetic characteristics when evaluated for allozyme diversity (Table 3). Measures of genetic diversity were low for *Lampsilis radiata* ($p = 7\text{--}36\%$, $H = 0.004\text{--}0.041$) and high for *L. cariosa* ($p = 64\text{--}82\%$, $H = 0.260\text{--}0.318$). Other freshwater bivalves showed intermediate levels of genetic diversity.

For the conservation biologist, genetically differentiated, geographically isolated populations are especially problematic. It is difficult to know whether they represent variation within a species or distinct species. This situation is widespread among North American unionids and is accelerating as habitat becomes increasingly fragmented. Avise et al. (1987) provide a powerful phylogeographic approach in which geography is overlaid on an evolutionary tree to provide a landscape perspective of relationships. Data available for *Quadrula*, *Pyganodon*, and *Amblema* populations illustrate this approach for freshwater bivalves.

Little genetic differentiation was detected for four sequential beds of *Quadrula quadrula* along a 31-km stretch of the Ohio River. Berg et al. (1997) attributed this homogeneity to movement of bivalves with host fishes. For more geographically isolated populations (range from 80 km to >1,500 km downstream) they observed variation in allozyme frequencies consistent with a model of isolation-by-distance and possibly selection in response to environmental conditions. Genetic characteristics of the widely

distributed and conchologically variable *Pyganodon grandis* were described by Liu et al. (1996a). Significant genetic differentiation was noted between populations of *P. grandis* from the South Platte River and Arkansas River drainages. Mulvey et al. (1997) used allozyme and DNA sequence data and a phylogeographic approach with *Amblema* species. Figure 3 shows the distribution of COI haplotypes. The data confirmed the genetic distinction between *Amblema neislerii* and *A. plicata* and identified some difference in haplotype occurrence between eastern and western populations of *A. plicata*. The authors recommended that conservation efforts be directed to *A. neislerii* which has a range restricted to the Apalachicola River drainage in Georgia and Florida and narrow habitat requirements. This study also identified a genetically distinct but conchologically cryptic form, *A. elliottii*, from the Coosa and Conasauga Rivers. Additional work will be required to determine the conservation status of *A. elliottii*.

Phylogenetic Reconstruction

Evolutionary history can be estimated through the study of contemporary taxa. However, as suggested by the studies above, contemporary taxonomy and phylogenetic analysis can be at odds because phenotypically defined categories are not necessarily monophyletic. Adamkewicz et al. (1997) present a phylogenetic reconstruction for bivalve mollusks, including the freshwater bivalves, *Elliptio complanata* and *Utterbackia imbecillis*, based on DNA sequences for the 18S rRNA gene. Rosenberg et al. (1994) used about 150 bp of the D6 region of the large RNA subunit (28S) to construct relationships among bivalve and gastropod mollusks. This study included 20 freshwater bivalves. The data supported the distinction between Margaritiferinae and Ambleminae as discussed by Davis and Fuller (1981) but did not recognize a distinct Anodontinae clade advocated by these authors. Lydeard et al. (1996) used DNA sequence data for the 16S rRNA gene to construct a phylogenetic hypothesis for North American Unionaceans based on 29 taxa (Fig. 4). These authors argued for the recognition

of families Margaritiferidae and Unionidae. The subfamily Anodontinae was clearly distinct from the remaining Unionidae; however, these data did not fully resolve relationships for the currently recognized Ambleminae and Lampsilinae. Additionally, these authors presented a previously unrecognized clade containing *Megaloniais* and *Quadrula*. Additional analyses of North American and global unionaceans are needed to clarify relationships and to serve as a basis for setting conservation priorities.

Hoch (1990) used allozyme and morphological data to generate phylogenetic hypothesis for 13 presumptive species of eastern North American *Anodonta*. Substantial revision was recommended with the three genetically differentiated clades given generic rank (*Anodonta*, *Pyganodon*, and *Utterbackia*). The molecular data suggested that ecophenotypic plasticity led to serious problems for traditional approaches to species determinations and evaluation of phylogenetic relationships. Umbo height relative to the hinge line, considered an important diagnostic characteristic, was shown to be misleading and did not identify monophyletic groups.

A cladogram derived from molecular genetic or other data can be used as a conservation tool. The terminal branches of the cladogram are extant taxa of potentially immediate conservation concern. Deeper branches indicate which groups are species-rich and which are species-poor. Although a controversial notion among conservation biologists, one strategy might be to focus conservation efforts on the smaller groups and assume that loss of one or two species from the species-rich clades would be less significant.

Bivalve-Fish Host Relationships

Conservation of freshwater bivalves is linked to fish conservation because of the complex life history and obligatory relationship of the glochidial stage. Regrettably, bivalve-fish host relationships are known for only about 25% of the North American bivalves and many proposed relationships are questionable (Hoggarth 1988, 1992). Failure to understand this critical life history relationship severely constrains conservation efforts. For example, the prob-

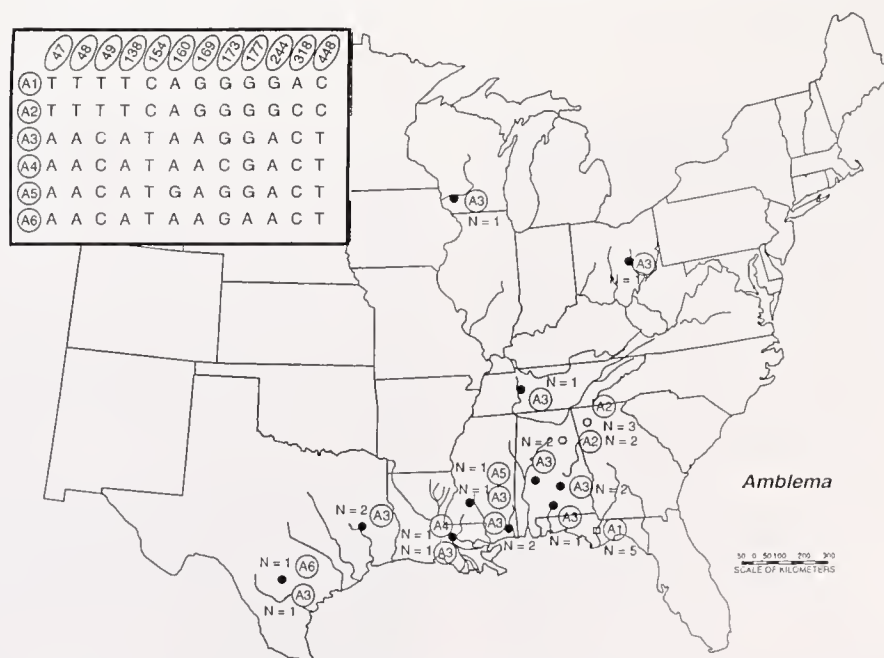


Figure 3. Distribution of haplotypes for the 16S rRNA gene for *Amblema plicata*, *A. neislerii*, and *A. elliottii*. (from Mulvey et al. 1997).

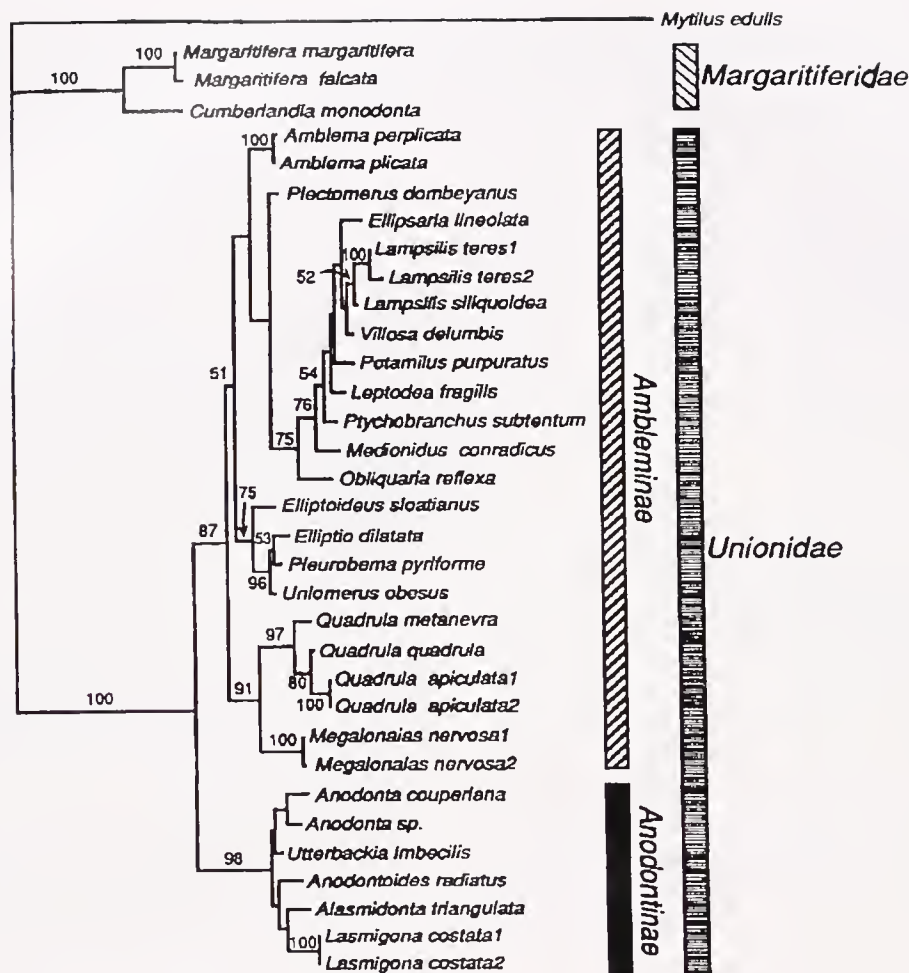


Figure 4. Phylogram generated from maximum parsimony analysis of 16S rRNA sequences for 29 species of North American unionaceans. (from Lydeard et al. 1996)

ability of success with in-situ preservation, translocation, or laboratory propagation would be greatly enhanced if reproductive requirements were known.

Their association with fish limits natural dispersal of unionids. The type and number of host fish utilized will affect dispersal and gene flow probabilities and, therefore, the likelihood of genetic divergence among populations. Kat (1984) argued that differences in patterns of genetic structure (determined with allozymes) in two widely distributed bivalves might be attributable to host fish relationships. *Elliptio complanata* commonly utilizes the yellow perch, *Perca flavescens*, as a host. Yellow perch are restricted to fresh or slightly brackish waters, display territorial behavior, and may be restricted to particular areas within a drainage. *Elliptio complanata* exhibited considerable differentiation in allozymes and morphological characteristics between drainages and even within drainages, as might be predicted from the limited dispersal associated with this host fish. In contrast, *Anodonta implicata* uses an anadromous host fish (alewife, *Alosa pseudoharengus*) which displays considerable movement within and, potentially, among drainages. *Anodonta implicata* populations exhibited relatively little genetic or morphological divergence among populations even at the extremes of their range.

Currently, most determinations of host fish suitability are done using laboratory exposures (Zale and Neves 1982). Other studies use morphological traits (e.g., glochidial shape, presence/absence

of hooks) to identify glochidia collected from fish taken in the field. Morphological traits often provide identification only to subfamily. A method that would allow glochidia to be identified to species after removal from fish would provide much-needed data on this important portion of the life history and significantly improve our ability to manage this fauna. To be useful, such a technique must accommodate many taxa (e.g., the Cumberland drainage is home to 87 unionid species) and also be able to distinguish among co-occurring congeners (e.g., at least five species of *Elliptio* occur in the Savannah River).

White et al. (1994) reported a molecular genetic technique that has promise for identification of glochidia taken from infested fish. They used RFLP patterns (ITS1 region) of encysted glochidia taken from fish and matched them to RFLP patterns of adult unionids to make identifications. However, the discriminating power of RFLPs was not sufficient below the level of genus for the 25 unionid species found in their French Creek, Pennsylvania study area. The authors suggested that DNA sequence data should be able to distinguish species. DNA sequences are unique to species or populations and could be used in habitats where many freshwater bivalves and fish co-occur.

Hatchery Populations

The rapid decline of many freshwater bivalve populations has led to increased efforts to rear critically endangered species in

hatcheries for eventual release to the wild. One goal must be the maintenance of genetic characteristics that maximize the probability of success when bivalves are repatriated. In the hatchery situation, two issues of concern lend themselves to a genetic approach: changes in allele frequency and loss of genetic variation due to drift and domestication during captivity. Genetic diversity may decline rapidly in captive populations because of small numbers of breeding adults, founder effects, and selection for hatchery-adapted stocks. Allozyme or DNA-based genetic markers provide convenient methods to characterize stocks at their establishment in the hatchery and to monitor stock integrity over generations of maintenance in the hatchery. An additional application of molecular genetic markers is the identification of unique stocks. Where genetically distinct populations (ESUs) have been identified, it is desirable to maintain separate gene pools as these may represent locally adapted gene complexes and their integrity would enhance the probability of success during repatriation.

Forensic Applications

Molecular genetic techniques have considerable potential for use in conservation law enforcement. Forensic applications include the identification of bivalve material that finds its way to commercial uses (Baker et al. 1996). PCR techniques especially are well suited to this problem. Only small amounts of tissue are needed and the many markers that can be developed make identification and determination of specimen origin very likely. For commercially exploited species transported across jurisdictions, this approach can lead to effective legal intervention. Molecular genetic data are currently being used to investigate allegedly poached washboards, *Megaloniais nervosa* (C. Lydeard, pers. com. to MM).

SIGNIFICANCE

Molecular genetic approaches are not a panacea for the conservation of freshwater bivalves. They are not appropriate for every species or every question, however, for some controversial issues such as the establishment of ESUs, they provide a powerful

tool. Knowledge of genetic population structure, evolutionary relationships, and taxonomic validity of names is essential in making appropriate recommendations for conservation of unionid diversity. Among the more than 300 species of North American freshwater bivalves, 31% are considered endangered, 14.5% threatened, and 24% of special concern (Williams et al. 1993). Therefore, the need to document patterns of genetic variation and species boundaries in unionids is urgent. On-going loss of suitable habitat and habitat fragmentation will likely accelerate the urgency. Studies of patterns and processes affecting genetic differentiation are critically needed to increase the effectiveness of conservation efforts directed toward freshwater bivalves.

In the face of huge ecological and demographic issues in the conservation of freshwater bivalves, does it make sense to spend limited resources on genetic studies? The answer is a resounding "yes." Although ecological and demographic considerations are essential, genetic data provide much to the decision-making process. Conservation goals for freshwater bivalves are twofold: to increase the likelihood of species survival, and to conserve ecological and evolutionary processes for the long term. Both goals necessarily require the maintenance of genetic diversity. However, a simple inventory or description of genetic characteristics is not sufficient to meet these goals. Freshwater bivalves exhibit differences in mode of reproduction (hermaphrodite versus gonochoristic), reproductive strategy (simple or elaborate packaging of larvae), and host specificity (narrow or wide). Therefore, genetic data must be coupled with knowledge of biogeography, life history, and ecological data to formulate management plans that meet conservation goals.

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ECOLOGICAL AND EVOLUTIONARY CONCERNS IN FRESHWATER BIVALVE RELOCATION PROGRAMS

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ABSTRACT Relocation of native freshwater bivalves has been used as a conservation tool primarily for restorative or supplemental reasons and to remove populations from the immediate impacts of construction projects. Though the presence of indigenous bivalves was most often used as an indicator of suitable habitat to receive relocated animals, subtle ecological and evolutionary differences between communities may have precluded successful relocation. We discuss the importance of maintaining genetic diversity within and among populations, the potential for spread of disease causing pathogens and parasites, and suggest approaches that could lead to improved relocation success, with the ultimate goal of maintaining the evolutionary lineages of freshwater bivalve species. Conservation efforts should strive to ensure that genetic variation is preserved to maintain the potential for future evolutionary change, and intervene as little as possible when managing the genomes of threatened species. However, when relocations are deemed necessary, every precaution should be taken to minimize the effects of gene drift and inbreeding depression by providing sufficiently large effective population sizes. Those involved in relocations should also exercise vigilance to avoid the pitfalls of outbreeding depression resulting from mixing divergent evolutionary lineages and the potentially catastrophic consequences of introduced pathogens. In addition, relocation attempts to supplement population size and maximize genetic diversity of historically small populations may lead to the loss of critical adaptive features including the relationship established with the parasitized host(s). Management efforts that focus on the protection of existing populations, and the discovery and protection of new populations of threatened taxa, may represent a more realistic conservation strategy than the creation of populations of unknown ecological and evolutionary potential.

KEY WORDS: Freshwater bivalves, genetic diversity, pathogens and parasites, relocation

HISTORY OF RELOCATION PROGRAMS

Relocation of native freshwater bivalves within and between drainages has been used as a conservation tool over the past several decades primarily for restoring or supplementing populations (Ahlstedt 1979; Sheehan et al. 1989; Jenkinson 1985; Koch 1993; Layzer and Gordon 1993) and to remove populations from the immediate impacts of construction projects (Jenkinson 1989; Trdan and Hoeh 1993). Cope and Waller (1995) recently reviewed the results of numerous relocation efforts and found that the majority included a monitoring phase to determine relocation success though most were monitored less than 1 y. Fewer than 20% were monitored for 5 y. Efforts to monitor the success and impact of relocation programs have focused primarily on determining survival rates of the relocated animals and relatively few efforts included evaluation of recruitment. Survival and recovery rates were generally low, often influenced by environmental factors and the species involved. Though most projects used the presence of indigenous bivalve species as an indicator of suitable habitat to receive relocated animals, subtle ecological and evolutionary differences between communities may have precluded successful relocation. Little evidence exists that past relocation programs considered identification and preservation of genetic diversity within a species or assessed the potential ecological health issues related to introduction of divergent genomes or disease agents to resident populations.

Presently, the invasion of North American waters by the zebra mussel (*Dreissena polymorpha*) poses a major threat to native freshwater bivalves and has resulted in a perceived need for relocation efforts. The impact in some areas is substantial and may be

the primary cause for the decline of bivalves in the Great Lakes (Schloesser and Napela 1994). As the zebra mussel advances, the prognosis for native freshwater bivalve populations is bleak, especially for populations of species considered to be threatened and endangered. In 1996, zebra mussel densities in the lower Ohio River exceeded 14,000 per square m, and mortality of native bivalves exceeded 30% (P. Morrison, Ohio River Islands NWR, USFWS pers. comm). Based on infestation rates seen in other rivers, the prognosis in areas of high infestation in the Ohio River is potential extirpation of endangered bivalve species, as well as an overall decline of the Ohio River native bivalve fauna. To help address the zebra mussel threat, salvage programs have been instituted that allow the removal of threatened and endangered bivalve species from zebra mussel-infested areas of major rivers. The urgency felt as a result of zebra mussel introduction limits the amount of time available to conduct research into aspects of native freshwater bivalve relocation.

Use of relocation and salvage programs as conservation tools must be carefully scrutinized. The impact on the ecological and evolutionary processes within species and associated communities should be investigated given the importance of these processes to long-term survival of species (Hamrick and Godt 1996) and the vulnerability of many freshwater bivalve species to extinction (Williams et al. 1993). Not considered in previous relocations was the role genetic diversity may play with regard to fitness of relocated animals (Hinch et al. 1986; Hinch and Green 1989; Iglesias et al. 1996) and the potential consequences of combining animals from disjunct populations. Furthermore, since most relocations have occurred within the same drainage, the presence of the fish host was often assumed prior to relocation. The literature revealed

few relocations (Sheehan et al. 1989; Jenkins on 1985; Layzer and Gordon 1993; Morgan et al. 1997) verified the presence of the host or attempted to relocate the host along with the bivalve species.

We will discuss the importance of maintaining genetic diversity within and among freshwater bivalves, and the likelihood for spread of disease causing pathogens and parasites to relocated or resident animals. Finally, we will suggest approaches that could lead to improved relocation success and ultimately help maintain the evolutionary potential of freshwater bivalve species.

GENETICS CONCERNS

Biological classification provides the fundamental basis for management decisions, yet effective management of imperiled species is often complicated because inadequate information exists for identification of species, subspecies, or distinct population segments (Avice and Nelson 1989). Morphological taxonomic methods are often inadequate to sufficiently differentiate groups along phylogenetic lines due to the lack of congruence of characters (Stepien and Kocher 1997). These characters, which can be phenotypically plastic, often fail to provide identification of closely related species or intraspecific groups (Avice 1994). Recent advances in the development of molecular biological techniques allow investigators to detect genetic variation at an unprecedented scale and have made available numerous genetic markers to evaluate evolutionary processes such as rates of change or constraints on change (Hillis et al. 1996). Concomitant with the advances in resolution has been the rise of phylogenetic systematics (or phylogenetics) to examine inter- and intrageneric relationships (Hillis et al. 1996, Ferraris and Palumbi 1996, Stepien and Kocher 1997) and intraspecific phylogeography, a coalescence of population genetics, phylogenetics, and biogeography (Bermingham and Moritz 1998). Thus, contemporary population genetics and phylogenetic studies have provided the essential ingredients (i.e., informative molecular markers) to achieve more enlightened evolutionary inquiries (Hillis et al. 1996).

Freshwater bivalves, which are among the most threatened animals in North America (Williams et al. 1993), present major taxonomic quandaries and other significant challenges to conservation biologists attempting to maintain ecological and evolutionary processes within and among populations. Bivalves are phenotypically plastic as both morphology and conchology are susceptible to environmental influences (Baker 1928, Galtsoff 1964, Johnson 1970). External phenotypes are not an infallible guide to the partitioning of genetic diversity and may not reveal true genetic relationships because the rate of change is not the same in all lineages and evolution may cause species to converge as well as diverge.

Freshwater bivalves possess a complex reproductive cycle requiring a host for successful transformation from a glochidial stage. It has been suggested that the dispersal pattern of the host is related to the level of genetic differentiation among bivalve populations (Kat 1983, Kat 1984, Kat and Davis 1984). Some bivalve species and even individuals within a population are hermaphroditic with the potential for simultaneous gonadal development and subsequent self-fertilization (Downing et al. 1989). The explicit impact of hermaphroditic reproduction or the host on genetic population structure of freshwater bivalves remains unknown.

Biologists recognize the importance of diversity within species and the potential for morphological, physiological, behavioral, or genetic diversification among populations (Meffe and Carroll

1994). Conservationists also appreciate that some component of the variation is genetically based and evolutionarily significant (Futuyma 1986, Meffe and Carroll 1994). However, relatively little information is available on the amount or distribution of genetic diversity present at any taxonomic level in freshwater bivalves. If a goal of conservation efforts is to permit the continued evolution of species (or any unit of management), then it is important to establish the genetic and taxonomic relationships among managed individuals or populations. Correct delineation of the unit of management is especially critical when the composition of a population is manipulated, whether by reintroduction from external stocks or by reestablishment of gene flow and migration patterns by the exchange of individuals from different populations.

A common assumption in conservation genetics is that higher levels of within-population genetic variation (i.e., heterozygosity) enhances the probability of a population's survival over evolutionary time (Avice 1994). In the context of a relocation program, genetic diversity may decline rapidly in bivalve populations as a consequence of severe bottlenecks if the number of breeding adults is small (i.e., due to founder effects). In small populations, gene frequencies change from generation to generation, even in the absence of selection, mutation, or migration. A gradual increase in homozygosity (i.e., an overall decline in genetic diversity) is a likely outcome for small populations due solely to chance effects (i.e., random genetic drift). The probability of small populations with reduced levels of genetic diversity surviving a stochastic event, such as an environmental perturbation, may be reduced. If relocation programs are based on small populations, genetic diversity may be lost at a rate inversely proportional to a subset of the actual viable number of adults (i.e., the effective population size) (Vrijenhoek 1996). For freshwater bivalves, the genetically effective population size is influenced by such factors as hermaphroditic reproduction, fertilization success, stochastic fluctuations in population size, and low densities of hosts.

Managers implementing freshwater bivalve relocation programs should be aware of factors that reduce the effective population size, increase homozygosity, and ultimately increase the risk for reduced fitness (i.e., inbreeding depression). Studies on experimental animals from wild populations (Templeton 1986, Allendorf and Leary 1986, Obrien et al. 1996) and zoological parks (Ralls and Ballou 1983) consistently confirm the ubiquity and magnitude of the effects of inbreeding depression. However, species differ markedly in their resistance to inbreeding depression. Numerous plants reproduce naturally by self-fertilization and those freshwater bivalve species that are hermaphroditic may innately resist inbreeding depression. In the absence of empirically derived data for freshwater bivalves, one must assume that inbreeding depression is a significant consequence of reduced population size in relocation programs.

Genetically diverse populations should be capable of adapting more successfully to biotic and abiotic changes in their environment. For example, individuals possessing two forms of a gene (i.e., heterozygous for a gene or gene product such as an enzyme) may perform better than individuals exhibiting a homozygous genotype. The mechanisms underlying the associations between heterozygosity and fitness are not well understood (Vrijenhoek 1994) and are the focus of much debate (Ohta 1981, Turelli and Ginzburg 1983, Ledig et al. 1983, Zouros and Foltz 1987). However, increasing evidence supports a positive correlation between heterozygosity and fitness as expressed by survival, growth, fertility, and disease resistance in plants and animals (Allison 1955,

Frelinger 1972, Mitton and Grant 1984, Allendorf and Leary 1986, Ledig 1986, O'Brien and Evermann 1988). Although few data comparing freshwater bivalves are available, a number of studies of marine bivalves have suggested a positive association between survival and heterozygosity during some portion of their life cycle (Allendorf and Leary 1986). Managers of remnant freshwater bivalve populations should be cognizant of the potential usefulness of heterozygosity-fitness correlations (Vrijenhoek 1996).

Relocation of bivalves between previously isolated populations may be a viable mechanism to supplement depleted populations and simultaneously combat inbreeding depression. However, there are potential problems associated with the mixing of discrete populations, including outbreeding depression (Mayr 1963, Wallace 1968, Templeton 1986, Leberg 1993), the loss of local adaptations (Lande 1995), and the spread of pathogens and parasites (Andrews 1980; see next section). Outbreeding depression refers to the phenomenon of fitness reduction in offspring following introgressive hybridization either in immediate hybrids or later generations due to the disruption of complex developmental pathways that have evolved to function in a coordinated manner (i.e., coadaptive genes) (Templeton 1986). Therefore, if genetically divergent bivalve populations are mixed, the resulting hybridization could disrupt coadapted gene complexes and increase the probability of continued population decline, especially during the initial stages of the relocation effort (Templeton 1986).

Evolution occurs as a result of successive change in the kinds and frequencies of genes that occur in populations, and it results in progressive increase in the adaption of organisms to their environment (Lande 1995, Lynch 1996). Attempts to supplement population size and maximize genetic diversity of historically small populations by relocation may lead to the loss of critical adaptive features including the relationship established with the fish host. In many host-parasite relationships genetics plays the primary role in host recognition and selection. Host recognition often is determined by chemical cues (Bush 1975) and the genes controlling host selection are involved in chemoreception. If the original (relocated) population and the receiving population are significantly divergent as to utilize different host species, the relocated population may be unable to sustain itself in the new environment.

DISEASE CONCERNS

Limited information exists in the scientific literature concerning diseases, or potential etiological agents of disease, in freshwater bivalves. Diseases caused by bacteria, viruses, parasites, and those of unknown etiology have been reported in marine bivalves, and the transfer of disease among marine bivalves is well documented (Andrews 1980; Lauckner 1983; Bower and Figueras 1989; Figueras 1989). Categories of pathogens that have been reported or suspected in marine bivalves include (1) bacterial, including *Rickettsiae* and *Chlamydiae*; (2) algal, which causes decreased filtering and larval settlement; (3) protozoans; (4) metazoans such as *Proctoea maculatus*, whose pathogenicity is varied depending on host species and environmental factors such as temperature; and (5) crustaceans including *Mytilicola* and *M. intestinalis* which have been the cause of high mortalities (Bauer et al. 1994).

Much can be learned from the experiences involving eastern oyster (*Crassostrea virginica*) relocation programs which illustrate the potential ecological and evolutionary consequences of bivalve relocations. To sustain commercial harvests, oysters have been

transplanted in coastal waters without much regard for adaptations of local races to the new environments (Andrews 1980). The extensive relocation of native oysters can consequently lead to genetic mixing and to the potential spread of diseases, with the impact on endemic communities often difficult to predict. Andrews (1980) reviewed introduction programs of oysters and provided vivid examples of the consequences of moving the species from one locality to another. Malpeque Bay disease in oysters was documented by Needler and Logie (1947). Oysters (*C. virginica*) from New England were used to supplement oyster production in Malpeque Bay at Prince Edward's Island, Canada. Within the first year of importing oysters, severe mortality occurred due to an unknown etiology and continued for a number of years. Though native oysters eventually developed resistance to the pathogen, the disease spread throughout the bay, to other bays and tributaries of mainland New Brunswick, with the pathogen still present in these systems. Imported oysters carrying the pathogen *Minchinia nelsoni* are believed to have caused over 90% mortality in oysters from the Delaware and Chesapeake Bays in the late 1950s (Rosenfield and Kern 1979). *Perkinsus marinus* from seed oysters transported from South Carolina is believed to have caused a disease in oysters in the Chesapeake Bay. These examples imply that populations of *C. virginica* may have evolved differing degrees of susceptibility to disease and pathogens, which would reflect genetic differences between these populations.

The general lack of information on diseases of freshwater bivalves reflects the minimal effort placed in this area of study because, aside from the use of shells of certain species for seed material for production of cultured pearls, they have not achieved the economic importance of marine bivalves. One study is available which describes a pustular disease in the freshwater species *Anodonta anatina* in the Vantaa River (Pekkarinen 1993), causing slow shell growth and even death, but the cause or etiological agent is unknown. Basic information, including the bacteria and viruses inherent in native freshwater bivalves, is often not available in the literature. Starliper et al. (1997) evaluated the native bacterial flora of freshwater bivalves from the Ohio River. They found that animals maintain a bacterial flora on average $1-3 \times 10^5$ cfu/g of soft tissue and although total cfu/g remained relatively stable, the bacterial flora changed within 24 h after the animals were placed in water different from their source water, likely related to the pumping rate of the species, environmental flora, and various environmental conditions.

A not so obvious consideration of relocations is prior exposure to pathogens. For example, bivalve species A is moved to a site with bivalve species B present. Species A is a pathogen carrier and could be innately immunologically tolerant, but species B has not been previously exposed and is more susceptible. If environmental conditions at the relocation site are conducive to survival of the introduced pathogen, survival of species B has now been jeopardized. The reciprocal of this situation may also occur, where species A is more susceptible to pathogens present at the relocation site.

Because of their anatomy, bivalves can harbor many water-borne organisms. Andrews (1980) presented historical evidence of the introduction of exotic invertebrates through oyster relocations. The oyster drill, *Urosalpinx cinerea*, was one of several exotics transported with the oysters that have had economic consequences on the oyster industry in Europe. The importation of *Crassostrea gigas* from the Pacific Ocean has resulted in the decline of at least two oyster species in Europe, notably the extirpation of the Por-

tuguese oyster in France (Andrews 1980). The potential for a disease or parasite to be spread to nonbivalve organisms has been documented between imported oysters and native crabs. Parasites found in the shells of imported oysters infested the native mud crabs *Eurypanopeus depressus* and *Rithropanopeus harrisi*, which scavenge dead oysters, and is believed to be the cause of their decline in Virginia (Van Engel et al. 1966). Bower and Figueras (1989) discuss the potential for transfer of pathogens during transplantation of bivalves, both among individuals or species of bivalves, and from bivalves to other animals. The marine bivalve *Mytilus edulis* can be a host of the oyster pathogen *Marteilia refringens* and potentially serve as a vector. A similar scenario could be envisioned with reference to the fish host of freshwater bivalves. Known fish pathogenic bacteria have been identified in marine bivalves (Lauckner 1983). Recently, *Flavobacterium columnare*, a pathogen to many species of warm and cool water fishes (Bullock et al. 1986), was isolated from a freshwater bivalve *Amblesoma plicata* taken from the Ohio River (Starliper et al. 1997). Relocation of freshwater bivalves without consideration for the potential of disease contagion could introduce the risk. This will be exacerbated in intensive culture-rearing facilities and at facilities rearing bivalves and fish.

Since fish are an integral component of the freshwater bivalve life cycle, the spread of disease to the host can significantly affect the ultimate success of bivalve relocation efforts. The survival of relocated bivalves depends on the presence of the host fish in this new habitat and their ability to successfully exploit the host. It is known that fish species and populations can develop innate resistance to certain pathogens through continued and natural exposure. Relocated fish may lack pathogen specific immunity, and therefore, be susceptible to pathogens in the new environment. The same could be true with innate resistance in relocated bivalve species.

Though zebra mussels are not considered pathogenic, per se, they are a good example of how freshwater bivalves could serve as a "vector" in a relocation effort. A recent example involved the collection of *Fusconaia ebena* in a zebra mussel-infested portion of the Ohio River. Current quarantine protocol (Chaffee 1997) calls for holding native bivalves in zebra mussel-free water for 30 days, at which time veligers should be visible. Collected *F. ebena* were initially scrubbed and examined for zebra mussels prior to quarantine. After 30 days, zebra mussels were visible, initiating another scrubbing, examination, and another 30-day quarantine. After the second 30-day quarantine, zebra mussels were again visible on the native shells. It is believed the zebra mussels were present in the hinge and crevices of the native freshwater bivalve shells and once they outgrew that location, they migrated out onto the shell where the space could accommodate further growth. This experience provided evidence for the potential and ease with which a nondesirable or exotic organism can be relocated with freshwater bivalves if sufficient quarantine protocols are not in place.

RECOMMENDATIONS FOR ENHANCING LONG-TERM RELOCATION SUCCESS

To maximize its effectiveness as a conservation tool, a freshwater bivalve relocation strategy should address two basic objectives: (1) it must provide for a high probability of long-term persistence of the species, and (2) it must do so with minimal impact

on other organisms in the ecosystem. To achieve these objectives, we propose that relocation should be based upon the potential for gene exchange between geographic populations (i.e., connectivity between bodies of water) and knowledge of and access to fish hosts. However, some degree of risk must be acknowledged under these conditions, as the potential for large-scale spatial genetic differentiation among geographic populations of bivalves over short distances has been documented (King et al. 1994). Relocation is not recommended until a direct evolutionary relationship (i.e., confirmed the presence of gene flow) is confirmed between the geographic populations and the host species is determined. Molecular genetic techniques are available for confirming the presence of gene flow among populations of freshwater bivalves. Freshwater bivalve species and their hosts have evolved together over time, therefore, the bivalve and its fish host should be managed as a unit. Thus it is critical that the host be determined for each bivalve geographic population, unless there are no barriers to fish movement between sites within the drainage.

Because of the dependence on a host (e.g., fish) for completion of their life cycle, additional research is needed to determine the identity of the host and the status of the host population at the proposed relocation site. Although the identity of the host(s) species is unknown for many freshwater bivalves, a strong correlation has been observed between bivalve diversity and fish diversity (Watters 1992). Thus, it seems possible (even probable) that a relationship exists between bivalve and fish distributions. If true, gene flow among geographic populations of bivalves may be highly dependent upon the obligately parasitized fish host. In an attempt to link freshwater bivalve genetics and population structure to the population structure of the host fish, Kat and Davis (1984) proposed that the dispersal pattern of the host species may explain the level of genetic differentiation among populations of a freshwater bivalve, *Elliptio complanata*, in Nova Scotia. In an ongoing study by King et al. (unpubl), DNA sequence variation indicates a lack of gene flow between tributaries of the same river and between northern and southern drainages supporting populations of *Lasmigona subviridis* in the Atlantic drainage. This finding may reflect utilization of behaviorally divergent or reproductively isolated fish populations or utilization of an entirely different species of fish host.

Relocation of a species between drainage systems within the species' range with a confirmed host species present is acceptable if the evolutionary relationships between the freshwater bivalve populations affected by relocation are known and the relationship with the host is determined and maintained following the relocation. In addition, a quarantine system should be in place to minimize the risk of transferring pathogens or unwanted organisms, such as the zebra mussel, into the receiving system. Relocation is unnatural, and probably inappropriate for some systems, because the current isolation of distinct populations is a consequence of natural climatic and geologic processes rather than anthropogenic disturbance. Artificial introgression between isolated populations will no doubt alter the traits that define the native forms. Indiscriminate mixing might disrupt local adaptations, lead to outbreeding depression, and introduce pathogenic agents.

To minimize the risk for introducing diseases, studies should be designed to identify pathogenic diseases in freshwater bivalves, including those caused by bacteria, viruses, and parasites. These studies should determine the potential for pathogens to be spread between bivalve species, as well as from bivalves to fish, and vice

versa. Surveys should be conducted of resident fish and bivalves at the relocation site to determine background levels of pathogens in these ecosystems. Susceptibility to diseases may vary between freshwater bivalve populations, therefore efforts must be made to determine that animals are pathogen free prior to relocation to reduce the risk of introducing the disease to areas where they may not currently exist. At a minimum, bivalves should be inspected and tested for certifiable fish pathogens before relocation. A well-designed monitoring program should document short- and long-term effects to the bivalve community. Pathogens may not only affect the survival of relocated or resident bivalves but also their overall fitness and reproductive potential.

Conservation of freshwater bivalves will likely encompass intensive culture for enhancement and broadening of natural communities, especially for threatened or endangered species. In conjunction with intensive culture of bivalves, genetic integrity and prevention of disease are concerns that will surface. Ensuring desirable outcomes will depend on husbandry practices that will correctly address these concerns.

Relocation outside the species' known geographic range, otherwise known as an introduction, does not constitute a conservation strategy and should not occur even if a recognized fish host from within the range is present. Community-level devastation resulting from the introduction of non-indigenous plants and animals has been well documented (Pimm 1991, Meffe and Carol 1994). Regarding aquatic systems, the extinction of native fishes of North American deserts precipitated by the introduction of predatory exotic fishes (Minckley and Deacon 1991) and the spread of nonindigenous mollusks, especially Asiatic clams (*Corbicula fluminea*) and zebra mussel (*Dreissena polymorpha*), represent some of the more striking examples of disastrous introductions.

Relocations to prevent deterministic extinction when something essential is removed (e.g., available habitat) or when something lethal is introduced (e.g., zebra mussels) are probably warranted even if the relocation criteria mentioned above are not satisfied. But the relocation should not increase the likelihood of normal environmental perturbations resulting in a stochastic extinction. The introduction of a small, effective population with or without the presence of a host fish, combined with all the stresses associated with the relocation process, may provide conditions that will increase the likelihood of a stochastic extinction (Soulé and Simberloff 1986).

SUMMARY

The maintenance of genetic diversity is a prerequisite to the conservation of ecological and evolutionary processes and thus, the ultimate survival of freshwater bivalve species. Factors affecting short-term survival (i.e., demographics) (Lande 1988, Neves 1997) should not be given priority over genetic considerations (Hamrick and Godt 1996) as the evolutionary potential (i.e., long-term viability) of any species depends upon the amount of genetic diversity present. If ecologically and evolutionarily discrete geographic populations (or species) of freshwater bivalves are perceived to be homogeneous, this has the potential to lead to severe lumping and to the dismissal of intraspecific groups. This has immediate implications for conservation biology because of the critical role taxonomy plays in the implementation of the Endangered Species Act (ESA), particularly regarding invertebrates. Freshwater bivalves and other invertebrates are not recognized at

the population level as units eligible for protection and management under the ESA. The ESA, as amended in 1978, grants protection to "any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife that interbreeds when mature." Invertebrate and plant populations that constitute an "important component in the evolutionary legacy of the species" can only be protected under the ESA if the entire species is listed or if the observed differentiation warrants a subspecific designation. Similarly, if a segment of the species' range warrants removal from the list, the entire species or subspecies must be removed from protection. If a goal of conservation biology is to preserve genetic integrity and evolutionary lineages, recognition and protection must be mandated for intraspecific differentiation within bivalves.

Any future freshwater bivalve relocation efforts should be designed as controlled multidisciplinary experiments directed at determining the appropriate effective population size, documenting the relationship between bivalve and host, and measuring the impact of the introduction on the entire bivalve and fish community. Though bivalve species can be maintained in different environments through continual addition of relocated and cultured animals, the goal of a conservation strategy should focus on identifying the number of individuals needed to produce self-sustaining populations. In relocation programs, the size and structure (sex ratio, age) of the population being relocated should be sufficient to provide for long-term viability and sustainability should no reproduction occur with the resident population.

Restoration or enhancement efforts should give earnest consideration to the ultimate goals of the restoration effort (e.g., self-sustaining populations), the ecology and genetics of any existing population, the complexity of the species' life cycle, and the taxonomic relationship of donor stock to the receiving population. Freshwater bivalve conservation should strive to ensure that genetic variation is preserved to maintain the potential for future evolutionary change, and intervene as little as possible when managing the genomes of threatened species. However, when relocations of freshwater bivalves are deemed necessary, every precaution should be taken to minimize the effects of gene drift and inbreeding depression by providing sufficiently large effective population sizes (probably hundreds of animals rather than dozens). In addition, those involved in such relocations should exercise extreme caution to avoid the pitfalls of outbreeding depression resulting from mixing different evolutionary lineages and the potentially catastrophic consequences of introduced pathogens.

Management efforts that focus on the protection of existing populations, and the discovery and protection of new populations, would probably represent a more realistic conservation strategy than the creation of populations with unknown ecological behavior and evolutionary potential. In addition, theoretical genetic models indicate that although small isolated populations rapidly lose within-population variation, overall genetic diversity may be retained better than in a single panmictic population. An alternative strategy could be to maintain ecologically and genetically healthy populations within their natural locales. However, if human activities are currently the primary cause of extirpation or isolation, rather than natural processes, the restoration of threatened or extirpated populations by introductions from outside sources can be viewed as a management strategy that attempts to preserve the evolutionary processes of gene flow and colonization (Carlquist 1974).

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NATIONAL MARINE FISHERIES SERVICE AND THE EVOLUTIONARILY SIGNIFICANT UNIT: IMPLICATIONS FOR MANAGEMENT OF FRESHWATER MUSSELS

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ABSTRACT The National Marine Fisheries Service (NMFS) published a policy on applying the definition of species under the Endangered Species Act (ESA) to Pacific salmon on November 20, 1991 (56 FR 58612). This policy states that a stock of Pacific salmon will be considered a distinct population, and hence a "species" under the ESA, if it represents an evolutionarily significant unit (ESU) of the biological species. NMFS has been implementing this policy for Pacific salmonids since it listed Snake River sockeye salmon in 1991. While genetic data play a central role in NMFS' ESU concept, they are not always available, and NMFS makes an effort to compile and evaluate available phenotypic, life history, and habitat information when conducting status reviews. Examples of how NMFS has used the ESU policy to delineate ESUs are provided. NMFS and the U.S. Fish and Wildlife Service (FWS) issued a joint policy on the recognition of distinct vertebrate population segments under the ESA on February 7, 1996. The concept of a distinct population segment can be applied to invertebrates in proactive efforts to protect species outside of the ESA. Genetic, phenotypic and life history data, and habitat characteristics can provide valuable information to managers of invertebrate species on which units to conserve in order to prevent ESA listings and recover species.

KEY WORDS: Genetics, conservation biology, management units, ESU, freshwater mussels, Pacific salmon, Endangered Species Act

INTRODUCTION

When the Endangered Species Act (ESA) was first passed in 1973, the term "species" was defined as "any subspecies of fish or wildlife or plants, or any population of such species." In 1978, attempts to make this definition more restrictive resulted in the current definition of species, "any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature." Though it is important to take actions to conserve distinct vertebrate populations before they need ESA protection, this is even more important for distinct invertebrate populations because they are not recognized by the ESA. In order to list an invertebrate species, National Marine Fisheries Service (NMFS) or the U.S. Fish and Wildlife Service (FWS), depending on jurisdiction, must determine that the species is threatened or endangered throughout its range. However, any policy developed to interpret "distinct population segments" should be useful in providing managers of invertebrate resources with a tool to determine appropriate management units for conservation, regardless of the limitation in the ESA. In this paper I discuss the evolutionarily significant unit concept developed by Robin Waples for Pacific salmon and adopted by the National Marine Fisheries Service. I describe how the ESU concept is used in delineating Pacific salmonid populations, and I speculate on how it might be used to manage and protect freshwater mussel populations.

Policy on applying the definition of species under the ESA to Pacific Salmon

With the 1990 petitions¹ to list specific salmonid populations (Snake River sockeye and chinook salmon) as threatened or en-

dangered under the ESA, NMFS felt an urgent need to define the term "distinct population segment." After publishing an interim policy and considering public comments, NMFS published a final policy on applying the definition of species under the ESA to Pacific salmon on November 20, 1991 (56 FR 58612). This policy states that a stock of Pacific salmon will be considered a distinct population, and hence a "species" under the ESA, if it represents an evolutionarily significant unit (ESU) of the biological species. A stock must satisfy two criteria to be considered an ESU: it must (1) be substantially reproductively isolated from other conspecific population units; and (2) represent an important component in the evolutionary legacy of the species. This policy is based on a NOAA Technical Memorandum prepared by Robin Waples (Waples 1991a). Waples (1991b) and Waples (1995) provide further elaboration on the ESU concept as it applies to Pacific salmon and conservation of biological diversity.

NMFS has been implementing this policy for Pacific salmonids since 1991. To determine whether a population satisfies the first criterion, NMFS has used tag return data to estimate stray rates, genetic indices to estimate levels of gene flow, observed recolonization rates, and physical or geographic features that are likely to act as barriers to migration. To determine whether a population satisfies the second criterion, NMFS considers important factors such as genetic, phenotypic, and life-history traits, and habitat characteristics. Though genetic data play a central role in NMFS' ESU concept, they are not always available. For example, when NMFS listed Snake River sockeye salmon as endangered, it relied entirely on phenotypic, life history, and habitat information. Genetic data collected subsequently confirmed that this population is an ESU. Waples (1995) discusses how the ESU concept was applied to Snake River sockeye, Snake River chinook, lower Columbia River coho, and Sacramento River winter-run chinook salmon,

¹When NMFS receives a petition to list species or populations under the ESA, it must initiate a process which includes: 1) a 90-day finding on whether or not the petition provides enough information to warrant a status review; 2) if a status review is warranted, a one-year determination on whether to propose a listing for the species or population; 3) if a

proposed rule is published, a final determination no later than one year after the publication date of the proposed rule on whether to list the species or population.

Illinois River winter steelhead, Umpqua River cutthroat trout, and Atlantic salmon.

In order to address the species definition under the ESA for all vertebrate species, NMFS and the FWS issued a joint policy on the recognition of distinct vertebrate population segments under the ESA on February 7, 1996 (61 FR 4722). This policy is similar to and consistent with NMFS' ESU policy. A population can be considered a distinct population segment if it is discrete and significant. In this policy, the first criterion of discreteness parallels the ESU's reproductive isolation criterion. Although political boundaries are not recognized by animals and plants, the discreteness criterion can also be met for policy purposes by international boundaries. The second criterion of significance parallels the evolutionary legacy criterion of the ESU definition.

The ESU policy in use

Although the number of petitions NMFS received annually did not increase, the number of populations being petitioned for listing increased annually from 1985 to 1994. This resulted in a reactionary, "piece-meal" process, which consumed an inordinate amount of staff and resources. Since 1994, NMFS decided to get ahead of the petitions and conduct coastwide status reviews² of Pacific salmonids in order to make comprehensive assessments. This was a good decision because soon after, NMFS received petitions to list all seven anadromous salmonid species from Washington to California. NMFS has used the ESU concept to determine that, in Washington, Oregon, Idaho, and California, there are seven ESUs of coho salmon (Weitkamp et al., 1995; NMFS Biological Review Team (BRT), 1996a), 15 ESUs of steelhead (Busby et al., 1996), four ESUs of chum salmon (BRT, 1996b), 15 ESUs of chinook salmon (three of which are already listed—Snake River fall, Snake River spring/summer, and Sacramento River winter-run (BRT, 1996c), 2 ESUs of pink salmon (Hard et al., 1996), and 6 ESUs of sockeye salmon (one of which is listed—Snake River) (BRT, 1996d). NMFS listed Central California Coast coho salmon, Southern Oregon/Northern California Coast coho salmon, and Oregon Coast coho salmon as threatened on October 31, 1996, May 6, 1997, and August 10, 1998 respectively (NMFS 1996, NMFS 1997a, and NMFS 1998f). NMFS also listed the Upper Columbia River and Southern California steelhead ESUs as endangered and the Snake River, Central California Coast, and South-Central California Coast steelhead ESUs as threatened on August 18, 1997 (NMFS 1997b). On March 19, 1998, NMFS listed the Lower Columbia River and Central Valley steelhead ESUs as threatened (NMFS 1998e). On March 9 and 10, 1998, NMFS also proposed to list 7 ESUs of chinook salmon (NMFS 1998a), one ESU of sockeye salmon (NMFS 1998b), two ESUs of chum salmon (NMFS 1998c), and two ESUs of steelhead trout as threatened or endangered (NMFS 1998d), as well as to redefine one currently listed ESU to include additional chinook populations (NMFS 1998a). NMFS designated some of the other ESUs that were not

²A status review involves gathering the best available scientific and commercial information on the petitioned species or population, determining which populations qualify as species under the ESA, assessing the status of the species, reviewing the past, current, and future threats to the species, and evaluating ongoing conservation efforts to determine whether the threats have been addressed.

Current Status of Steelhead

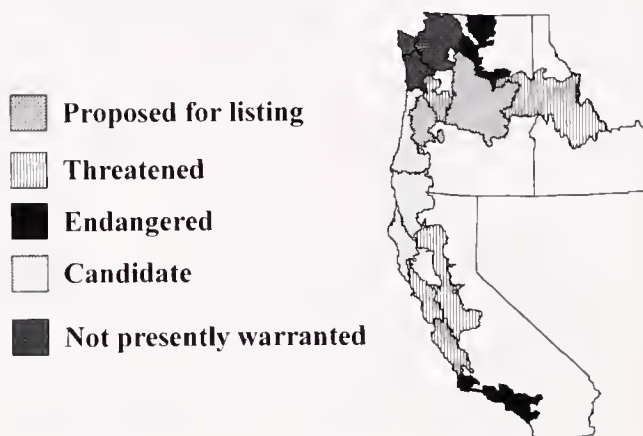


Figure 1. Status of steelhead trout evolutionarily significant units from Washington, Idaho, Oregon, and California.

proposed for listing as candidate species³ because of remaining concern over their status, and it determined that the other ESUs did not warrant a listing at this time.

Status reviews involve the collection of all available ecological and genetic data. Ecological data include run timing, distribution and abundance, size and age composition, occurrence of other fauna and flora, geography, hatchery influences, harvest influences, incidence of dams and other man-made barriers, and any other unique characteristics that can be observed. NMFS sends draft status reviews to comanagers and experts in the field for review to ensure that the best available scientific information has been obtained and analyzed appropriately before publishing the final status review.

During our coastwide steelhead status review, NMFS collected information on two major genetic groups recognized in North America—the inland and coastal groups. ESUs 13–15 are inland ESUs, and the rest are coastal (Fig. 1). NMFS also collected data for two basic reproductive ecotypes—the stream-maturing and the ocean-maturing. Inland steelhead of the Columbia River Basin are commonly referred to as either A-run or B-run, depending on when they enter the mouth of the river. NMFS found that the half-pounder⁴ life history form was reported only in the Rogue, Klamath, Mad, and Eel Rivers of southern Oregon and northern California. Rainbow and redband trout are the non-anadromous forms of *Oncorhynchus mykiss*, and NMFS determined that they were

³NMFS' candidate species list includes any species being considered by the Secretary for listing as an endangered or a threatened species, but not yet the subject of a proposed rule. NMFS conducts a review of the status of each candidate species to determine if it warrants listing as endangered or threatened under the ESA. FWS' new definition of candidate species is "those species for which the FWS has on file sufficient information to support issuance of a proposed listing rule."

⁴Half-pounder is a life history trait of steelhead exhibited in the Rogue, Klamath, Mad, and Eel Rivers of southern Oregon and northern California. Following smoltification, half-pounders spend only 2–4 months in the ocean, then return to fresh water. They overwinter in fresh water and emigrate to salt water again the following spring. This is often termed a "false spawning migration," as few half-pounders are sexually mature (Busby et al., 1996).

part of the ESUs. Because FWS implements the ESA for resident species, NMFS discussed with FWS whether or not to include these non-anadromous forms when it made determinations on the 10 ESUs proposed for listing. FWS preferred not to include resident forms with the listing determinations at that time.

The ESE concept and freshwater mussel conservation

The National Research Council (NRC), in its 1995 report titled, "Science and the Endangered Species Act," supported the recognition of scientifically identified evolutionary units for conservation purposes. The NRC also believed that recognition of distinct population segments would be appropriate for invertebrate species. However, the ESA is clear that only vertebrate distinct population segments can be considered species. In fact, congressional intent when defining vertebrate species to include distinct population segments was that this authority be exercised "sparingly and only when the biological evidence indicates that such action is warranted." (Senate Report 151, 96th Congress, 1st session). Because of the huge number of invertebrate species when compared with vertebrate species, Congress did not believe it would be appropriate to include population segments as species. The fact that these animals are not as charismatic as the spotted owl or the Pacific salmon most likely played a role in this decision. Some efforts have been made during the ESA reauthorization process to include invertebrate populations in the species definition again, as they were in the 1973 version, but in view of the drastic changes being contemplated by certain draft bills, it is unlikely that this definition will become more encompassing.

Despite the fact that the ESA limits the consideration of distinct population segments as species to vertebrates, the ESU concept can be applied to invertebrates in proactive efforts to conserve species not listed under the ESA. As Waples (1995) states "Outside the ESA, conservation efforts might be guided by any of several alternative contexts for interpreting evolutionary significance The key factor is how conservative one wants to be (or can afford to be) in attributing evolutionary significance to a biological unit" (page 23). Genetic, phenotypic and life history data, and habitat characteristics can provide valuable information to managers of invertebrate species on which units to conserve in order to prevent ESA listings and recover species.

Ecological and genetic data are invaluable regardless of whether populations can be protected under the ESA. Armed with data that delineate separate populations within a species, resource managers can determine where to concentrate conservation efforts and manage harvest accordingly, or avoid habitat damage in critical areas, and hopefully, prevent species from becoming extinct. As with Pacific salmon, freshwater mussel populations tend to be locally adapted to the areas they inhabit as they begin to diverge and become reproductively isolated from other populations (Davis and Fuller 1981). Freshwater mussel populations could be delineated based on life history characteristics, distribution, morphometrics, disease resistance, protein electrophoresis, and DNA differentiation.

As Dizon et al. (1992) states in his analysis of the stock concept for marine mammals, "Because of ecological phenomena such as predator-prey interactions, the existence of keystone species and species guilds, etc., a population may be important beyond its qualifications, or lack thereof, as an ESU; these factors must also be considered in deciding what should comprise a management

unit." (page 31) Since freshwater mussels have glochidial larval stages that in some species are dependent on a specific host fish, the specific host fish (or fishes) could be another factor to consider in delineating populations. Partial protection could be afforded these mussels by protecting the population of host fish under the ESA (as long as the population is reproductively isolated and represents a significant component in the evolutionary legacy of that species and the fish population is actually threatened or endangered). Because of the fish population's importance as a host to a dwindling mussel population, at least one life stage of the mussel species would be protected by protecting the fish population. Even without official protection, knowledge of the biological unit that constitutes a distinct population segment would help managers make day-to-day decisions regarding reintroduction efforts or viability of populations.

Systematists play a crucial role in the level of legislative protection afforded to shellfish. For instance, if data become available to indicate that a number of previously defined shellfish species should actually be classified as one taxonomic species, this has implications of whether the species can be protected under the ESA. If two of these previously defined species were in poor enough condition to be listed under the ESA, but then it was determined that they are only populations of a more abundant species, listing under the ESA would no longer be warranted because individual population segments of invertebrates cannot be listed. Hoeh and Gordon (1996) disputed Stiven and Alderman's (1992) conclusion, based on genetic distance criteria, that *Lampsilis radiata* and *L. radiata conspicua*, and perhaps *L. fullerkati* as well, should be considered simply as allopatric populations of *L. radiata*. Hoeh and Gordon (1996) claimed that the analysis was not rigorous enough, and since North American unionid populations are declining rapidly, the conservative and scientifically justifiable approach would be to continue to recognize the two latter species as taxa distinct from *L. r. radiata*. Dizon et al. (1991) notes that "Ideally, populations should be surveyed and phylogeographically classified *before* situations demanding management decisions arise." (page 32)

Allendorf et al. (1997) proposed a method of prioritizing Pacific salmon stocks for conservation based on both the risk and consequence of extinction. They highlighted the importance of genetic information by stating, "Genetic variation is the basis for the evolutionary legacy of a species—the unique pattern of its geographical distribution, as well as the morphological, physiological, and life-history variation that history has imposed on genetically differentiated populations or groups of populations within that species" (page 144). The questions they ask are the same ones that NMFS asks about each Pacific salmonid population as it determines whether or not it warrants species recognition under the ESA. A similar framework could perhaps work for prioritizing freshwater mussel populations for conservation. Ideally, proactive conservation efforts directed at populations of mussels could reverse a decline in abundance or distribution, resulting in fewer species listed under the ESA.

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NATIONAL STRATEGY FOR THE CONSERVATION OF NATIVE FRESHWATER MUSSELS

Prepared by: The National Native Mussel Conservation Committee,
June 1, 1997.

HISTORY OF THIS DOCUMENT

On April 1995, representatives from several federal and state natural resource agencies, the commercial mussel industry (Shell Exporters of America), academia, and The Nature Conservancy met to discuss freshwater mussel declines and gather information on freshwater mussel trends, research, and recovery activities (Appendix I). As a result of the magnitude and immediacy of the nationwide threats to the freshwater mussel fauna, the group agreed that a coordinated effort of national scope was needed to prevent further mussel extinctions and population declines.

To address this need, the group decided to (1) draft a National Strategy for the Conservation of Native Freshwater Mussels (National Strategy) and (2) establish a national ad hoc committee with broad-based representation from state, tribal, and federal agencies, the mussel industry, private conservation groups, and the academic community to help implement mussel conservation at the national level. A draft National Strategy was presented at the second Symposium on the Conservation and Management of Freshwater Mussels organized by the Upper Mississippi River Conservation Committee, in St. Louis, Missouri in October 1995. Comments received at and subsequent to the symposium were incorporated into another draft dated September 16, 1996. The September 1996 draft was presented at a February 1997 meeting of the newly formed National Native Mussel Conservation Committee in St. Louis, Missouri. Comments from the February 1997 meeting have been incorporated into this current document.

STATUS AND ROLE OF NATIVE FRESHWATER MUSSELS

The world's greatest diversity of freshwater pearly mussels, nearly 300 species, reside in the continental United States (Turgeon et al., 1988). However, within the last 50 years this rich fauna has been decimated by impoundments, sedimentation, channelization and dredging, water pollution, and, more recently, the nonindigenous zebra mussel (*Dreissena polymorpha*) (Neves, 1997). Approximately 67% of freshwater mussel species in the United States are vulnerable to extinction or are already extinct; more than 1 in 10 mussels may have become extinct during this century (Williams et al., 1993; Master et al., 1998).

Freshwater mussels are a renewable resource, providing significant ecological and economic benefits to the nation. They are ecologically important as a food source for many aquatic and terrestrial animals; they improve water quality by filtering contaminants, sediments, and nutrients from our rivers; and because they are sensitive to toxic chemicals, they serve as an early-warning system to alert us of water quality problems. In recent years the annual value of shells to the mussel shell industry has been estimated at \$40-\$50 million dollars. The mussel shells are used in the cultured pearl and jewelry industries, and the shell harvest provides employment to about 10,000 residents, primarily in the Mississippi River basin.

CONSERVATION STRATEGY GOALS

The goal of this National Strategy is to conserve our nation's freshwater mussel fauna and ensure that the ecological and eco-

nomic values to society are maintained at a sustainable level. Specifically, the purposes of this document are to (1) identify the research, management, and conservation actions necessary to maintain and recover the mussel fauna; (2) increase government and public awareness of the plight of these animals and their essential ecosystems, and garner support for species and habitat protection programs; and (3) foster creative partnerships (working and funding) among federal, state, tribal, and local governments and the private sector to restore the mussel fauna and environmental quality to our rivers.

Identification of Specific Problems, Goals, and Strategies

In order to conserve and restore native freshwater mussels, the National Strategy has identified a number of conservation needs or problems. Tasks or strategies designed to address the problems are subsequently enumerated, and when implemented, will direct the successful conservation of freshwater mussels.

The following problems, goals, and strategies have not been prioritized. The intent is to provide a list of strategies and allow each agency or organization to prioritize and choose the strategy(s) that best fits its own mission, funding, and expertise. However, Appendix II provides a list of ranking criteria to assist in ranking specific projects.

PROBLEM 1: There is no coordinated national strategy for the conservation of freshwater mussel resources.

GOAL: Increase coordination and information exchange among entities that study, manage, harvest, conserve, or recover native freshwater mussels.

STRATEGIES

1.1 Establish a National Freshwater Mussel Ad Hoc Committee to coordinate national mussel conservation activities¹. This ad hoc committee (Committee) should be comprised of one individual appointed by each of the following entities: the Service, Tennessee Valley Authority (TVA), U.S. Geological Survey (USGS), U.S. Army Corps of Engineers (Corps), U.S. Forest Service (USFS), National Park Service (NPS), Environmental Protection Agency (EPA), each state and tribe with significant mussel resources, the commercial mussel industry, the conservation community, and academia. The Committee will perform the following tasks:

1.1.1 Identify entities that study, manage, harvest, conserve, or recover mussel populations; solicit their support; and foster partnerships in mussel conservation. The Service, NBS, TVA, Corps, and several state natural resources agencies, the Great Lakes Indian Fish & Wildlife Commission, and the commercial mussel industry assisted with the development of this National Strategy (see Appendix I). However, there are numerous other individuals and agencies that are currently conducting research

¹The National Native Mussel Conservation Committee was established in February 1997 including an executive subcommittee to direct activities.

and management on freshwater mussels. Potential partners in the mussel conservation effort should be informed of the National Strategy and encouraged to join in its implementation (see strategies under Problem 2 and 10).

- 1.1.2 Develop and implement effective mechanisms to disseminate information on the progress of the mussel conservation effort to cooperators and interested parties. It is important that information on the conservation effort be readily available to all interested parties. This prevents duplication of effort and allows for better coordination of conservation activities. For example, the Tri-annual Unionid Report, compiled and circulated by Richard Biggins of the U.S. Fish and Wildlife Service, Asheville Field Office, Asheville, North Carolina, provides an existing mechanism to disseminate current information on mussel conservation. Copies may be obtained by calling 704/258-3939 or over the internet (<http://www.inhs.uiuc.edu/cbd/collections/mollusk.html/TUR>). Information exchange is also being facilitated by the development of Internet sites, including a unionid listserver (UNIO—to subscribe send an e-mail message to: Majordomo@lists.umbc.edu) as well as numerous web pages including the Illinois Natural History Survey Mollusk Collection (<http://www.inhs.uiuc.edu/cbd/collections/mollusk.html>). The Committee should review currently available information exchange mechanisms and develop additional options as needed.
- 1.1.3 Provide guidance for the mussel conservation effort. As representatives of the national mussel conservation community, the Committee will meet on an annual basis to review the current status of the collective mussel conservation effort. Each Committee member should submit an annual report to the Committee outlining his group's projects. Based on their discussions, the Committee should develop and circulate an annual appendix to this National Strategy. This report would (1) provide a brief overview of the status of the conservation effort; (2) summarize important research and management results; (3) identify any new problems and strategies; and (4) suggest direction for future research and management initiatives.
- 1.1.4 Coordinate a thorough search and summary of the current knowledge of basic biology, population characteristics, and habitat requirements of mussels. Much of the information that exists on freshwater mussels is scattered throughout various professional journals, government publications, unpublished research projects, museum records, and observation records of numerous individuals. If this information could be consolidated into a computerized annotated bibliography, the mussel conservation community and other interested individuals would have ready access to current knowledge to help expedite the conservation effort.
- 1.1.5 Serve as the primary advocate for the implementation of the National Strategy. Members of the Committee should act as primary advocates within their agency/organization for educating the conservation community, their respective agencies, and the general public about this mussel conservation effort.
- 1.1.6 Appoint a technical committee (four to six members) of

mussel researchers. These individuals would have specific mussel research or related expertise and would be available to review research proposals and reports, provide technical assistance, develop draft national standards for conservation techniques such as sampling, population augmentation, introductions, propagation and quarantine facilities/management, and make technical recommendations to the Committee.

- 1.1.6.1 Develop a standardized mussel sampling regime that can be used throughout the country. Mussel sampling techniques must be adapted to each situation based on available funding, the expertise of collectors, and environmental conditions. Therefore, it is difficult to standardize a sampling protocol. However, by standardizing some aspects of the sampling protocol, mussel biologists would be better able to compare data among sites and among collections at the same site (see Strategy 4.1).

- 1.1.6.2 Develop a standardized mussel die-off response procedure. Many mussel populations have experienced die-offs, and the cause(s) of most events has not been determined. It is unlikely that the cause(s) of all future die-offs can be ascertained, but a standardized die-off response procedure would help resource managers and commercial mussel fishermen respond in a more timely manner and collect the critical information and samples needed by researchers.

- 1.2 Foster and create new partnerships and facilitate the development of formal agreements (e.g., memorandums of agreement) among government agencies and private entities to help implement this National Strategy. The mussel conservation community is small and, by itself, cannot significantly alter the factors that threaten this faunal group. However, most of the strategies that benefit mussels and their habitat quality also significantly benefit other aquatic fauna and resource user groups (commercial mussel industry, sport fisheries, water supply industry, canoeists, birders, etc.). Partnerships with other entities are essential to the success of this mussel conservation program, and these partnerships should be actively pursued (see strategies listed under Problems 2 and 10).
- 1.3 Foster and create cooperative ventures with academic institutions and the private sector to address specific research, information, and conservation needs (see strategies listed under Problems 2–10).

PROBLEM 2: Quality mussel habitat continues to be degraded and lost.

GOAL: Protect and reverse the decline of quality mussel habitat.

STRATEGIES

- 2.1 Use information gathered under Strategies 4.1, 4.2, and 4.3 to identify important mussel resource areas and develop programs to conserve and recover these key areas. The magnitude of the mussel conservation challenge is great, but the resources available for mussel conservation are small. Managers should concentrate their efforts, within their area of responsibility, on those key habitats, research programs, and protection/enhancement activities that will achieve the greatest benefit to mussel conservation. Those few stream reaches that still

harbor diverse mussel populations should be protected from further habitat degradation to the extent possible. It is much more cost-effective to protect existing quality habitat than to restore degraded habitat.

- 2.1.1 Identify and inform potential partners of important mussel sites and develop cooperative agreements to conserve and recover mussel communities. It is essential for the success of this National Strategy that potential partners understand the importance of the resource at risk, how the conservation program will benefit mussels and other biota, and how ecosystem recovery will benefit other user groups.
- 2.1.2 Use existing federal, state, tribal, and local laws and regulations to protect mussel resources. There are many environmental laws and regulations that, if fully implemented, could provide better protection for mussel resources. Use existing information and information generated under Strategies 3.1.3.1, 4.1, 4.2, 4.3, 5.1, 5.2, 5.3, 5.4, and 5.5 to help ensure that mussel populations receive the full protection provided under existing laws.
- 2.1.3 Encourage federal, state, tribal, and local government entities to use their authority to review their activities for actions and alternatives that protect and recover key mussel habitats and communities. Many agencies, although not bound by law, have modified their projects and programs and even initiated distinct programs that benefit aquatic resources, including mussels. Some agencies have specific programs and funding targeted for use on projects to protect and recover aquatic resources. These agencies should be recognized for their efforts and encouraged to review their activities for potential adverse impacts to mussel habitats and to communities and avoid or minimize these impacts.
- 2.1.4 Encourage industry to review their activities for actions and alternatives that could protect and recover key mussel habitats and communities. Many environmentally conscientious industries implement habitat protection and enhancement programs on their land. Contact the appropriate industries, increase their awareness of the mussel resources subject to their activities, and assist them with improving their stewardship efforts, which will benefit both them and the downstream riverine habitat.
- 2.1.5 Encourage local landowners to review their activities and, when feasible, provide financial (e.g., Service "Partners for Wildlife" funds) or other incentives to the landowners to protect and recover key mussel habitats and communities. Many environmentally conscientious landowners implement habitat protection and enhancement programs on their land. Other landowners might be willing to conserve habitat if they had the necessary information or were provided with incentives. Investigate and implement new and innovative approaches to encourage landowners to protect aquatic resources. Develop a means to recognize the efforts of cooperating landowners.
- 2.1.6 Encourage conservation organizations, universities, schools, civic groups, and other organizations to assist in the protection and recovery of key mussel habitats. Substantial support and assistance for restoration efforts is available from many environmental groups and other

organizations. Their support should be encouraged and formally recognized.

- 2.1.7 Encourage conservation organizations (e.g., TNC, land trusts or other land protection organizations) and agencies to acquire key habitats to protect freshwater mussels.
- 2.2 Develop a list of case studies that identify and summarize successful habitat restoration and protection projects and make the information available to the mussel conservation community. Several habitat restoration projects are underway to protect significant mussel resources. A list and description of these projects and the addresses of project managers would be helpful in the initiation of new projects.

PROBLEM 3: The basic life history, reproductive biology, ecology, and habitat requirements of most mussels are unknown.

GOAL: Increase fundamental knowledge of basic biology and habitat requirements of mussels so that managers can more effectively conserve and manage our mussel fauna.

STRATEGIES

- 3.1 Initiate studies on life histories, population dynamics, and environmental requirements to obtain information necessary to effectively manage mussels. Unlike many other animal species, little is known about the basic biology and habitat requirements of most mussel species. The lack of basic knowledge hampers conservation efforts. The following list identifies some specific research needs.
 - 3.1.1 Fish host identification.
 - 3.1.1.1 Determine the specific fish hosts for mussel species in need of management.
 - 3.1.1.2 Determine the host fishes' biological needs and their population sizes necessary to support mussel reproduction and population viability.
 - 3.1.1.3 Determine the extent and mechanism of the immune response of host fish to glochidia.
 - 3.1.2 Mussel reproductive biology.
 - 3.1.2.1 Determine age and size at earliest maturity, peak reproductive years, fecundity, and reproductive longevity.
 - 3.1.2.2 Determine the period of spawning and gravidity as well as spawning and settling sites.
 - 3.1.2.3 Determine the level of recruitment needed for species survival and long-term viability.
 - 3.1.2.4 Determine the frequency of successful recruitment in native habitats.
 - 3.1.3 Mussel habitat requirements.
 - 3.1.3.1 Determine species-specific physical and chemical habitat requirements (e.g., substrate, flow, temperature, dissolved oxygen, hardness, pH, and alkalinity) for adults and juveniles.
 - 3.1.4 Mussel population dynamics.
 - 3.1.4.1 Conduct studies to determine the impacts of diseases, parasites, predation, and harvest on mussels and how these factors affect mussel population demographics.
 - 3.1.4.2 Determine population size and age class structure necessary to maintain a long-term viable population.
 - 3.1.4.3 Determine the demographics of representative mussel populations and the extent of natural

variations in recruitment; attempt to define what constitutes a healthy mussel population.

- 3.1.4.4 Evaluate the effectiveness of current harvest regulations in sustaining viable populations of both sensitive and commercial species.

PROBLEM 4: Knowledge of the current distribution and health of mussel populations is lacking, and much of the historic distributional data are not readily available.

GOAL: Increase knowledge of the status and trends of native mussel populations so that resource managers and administrators can better determine the species and populations most at risk and which populations could be managed for sustained commercial harvest.

STRATEGIES

- 4.1 Increase sampling effort to determine location, density, species composition, and status of existing mussel communities. Many rivers need basic or current survey information. Knowledge of the condition and location of mussel resources is critical to understand a species' status and develop proper management. The use of the standardized sampling regime to be developed under Strategy 1.1.6.1 is encouraged.
- 4.2 Gather historic mussel distribution data and make it more readily available. Many historic collections exist in museums, universities, and private collections. However, some specimens have been misidentified, and many of the collections have not been catalogued or the data are not readily available. This historic information is critical to understanding the current status of many mussel populations. The information also may be useful for identifying potential reintroduction sites and locating unknown populations.
- 4.3 Gather information on the occurrence and abundance of mussel stocks that have value for the commercial mussel industry and tribal subsistence. Some mussel populations, if properly managed, can provide a sustainable harvest with little or no impact on sensitive mussel species. Populations that could sustain a managed harvest should be identified and evaluated. Information gathered under Strategy 3.1.4.4 should be used to develop harvest management guidelines.
- 4.4 Develop a central database on the status and location of native mussel populations. Information should be categorized based on USGS hydrologic unit maps and mapped using GIS. The database can be used to track mussel populations and should include absence data.
- 4.5 Develop a mussel distributional atlas. In the early 1980s the Service funded the production of an Atlas of North American Freshwater Fishes (Lee et al. 1980). This document provides a distribution map for all North American freshwater fishes and includes information on the species' habitat and biology. The fish atlas has been a valuable tool for fisheries managers and biologists; a similar atlas on native mussels would benefit mussel conservation efforts.
- 4.6 Encourage the use of molecular genetics techniques to help identify mussel species. Historically, mussels have been described primarily on the basis of shell characteristics. This method has been very reliable, and there is little question regarding the taxonomic distinctiveness of most mussel species. However, molecular genetic analysis has shown that some species are comprised of complexes of distinct species (Kat, 1983a; Kat, 1983b; Lydeard et al., 1996; Mulvey et al.,

1997). Thus, some species believed to be widespread may be unknowingly lumped with species that are rare and in need of protection. Molecular genetic research should help clarify the taxonomic relationships within these complexes.

PROBLEM 5: Habitat alterations, water quality degradation, and other anthropogenic factors continue to negatively affect mussels, but poor documentation exists as to how and at what levels such perturbations are realized.

GOAL: Determine how various perturbations impact mussels and their habitat, and provide managers with the information needed to minimize or eliminate threats and protect quality mussel habitat.

STRATEGIES

- 5.1 Determine how and to what extent various habitat alterations affect mussel species and populations. The impacts to mussels from habitat alterations, such as the impounding and dredging of mussel beds, are fairly well understood. However, the links between the decline or loss of many mussel populations and the causative agent(s) are unknown. Research is needed to determine how and to what extent the following factors affect mussels (this list is not intended to include all of the potential mussel perturbation agents that need research): (1) increased siltation; (2) pesticides, herbicides, and fungicides; (3) stream-flow modifications; (4) wastewater discharge of various pollutants and subsequent sediment loading; and (5) modifications in water temperature, dissolved oxygen levels, nutrients, and pH. A better understanding of how environmental factors affect mussels will enable resource agencies to better manage and conserve mussel communities.
- 5.2 Determine if current water quality criteria protect all life stages of freshwater mussels. Bioassays should be conducted to evaluate the sensitivity of all life stages of mussels relative to the sensitivities of standard bioassay organisms. Surrogate species should be selected to be protective of most sensitive mussel species or appropriate buffers should be built into protective criteria models.
- 5.3 Determine if current "Best Management Practices" (BMP) protect mussel populations and their habitat. Great strides have been made in the development and implementation of BMPs for agriculture, silviculture, road and bridge construction, and other activities, and these practices have benefitted aquatic resources. Research is needed to determine if these practices adequately protect mussel populations and how they might be modified to be more effective. Information is also needed about the degree of voluntary compliance with BMPs.
- 5.4 Determine if current laws and regulations protect freshwater mussels. Many existing laws and regulations are aimed at protecting aquatic resources. However, information is needed to determine if they provide sufficient protection for rare mussels.
- 5.5 Review early literature to determine what historic factors may have caused the decline or extirpation of mussel populations. The loss or decline of some mussel populations in specific rivers is the result of historic rather than current conditions. A review of historic literature may reveal the reasons for a river's present lack of mussels. If the original cause of the loss has been eliminated or minimized, mussel reintroduction may be feasible.

5.6 Develop biomonitoring protocols using freshwater mussels to complement fish and other macroinvertebrate biomonitoring protocols presently used to evaluate the integrity of a stream. Fish and macroinvertebrate biomonitoring protocols have been developed to score and rank lotic systems for their health based on numbers and presence of sensitive species. Freshwater mussels are a very logical monitoring component for the biotic health of a system since they are generally long-lived and sedentary. This would provide valuable information for linking environmental threats to presence or absence of specific species, more adequately assess the integrity of streams, and provide a valuable tool to biologists and resource managers.

PROBLEM 6: The invasion of zebra mussels poses a new and significant threat to the continued existence of many native mussel species.

GOAL: Develop management options to eliminate or reduce the threat of zebra mussels to native mussels.

STRATEGIES

Note: Any new zebra mussel initiatives should be coordinated with other organizations (e.g., Sea Grant) that are already significantly involved with this species.

- 6.1 Develop predictive models on the spread of zebra mussels and their likely impact on native mussels. Zebra mussels have devastated native mussel populations in the Great Lakes (O'Neill and MacNeill, 1991; Kelch, 1994; Taylor and Kerschner, 1995), and they have now invaded inland rivers where they are likely to affect important commercial mussel resources and protected species. Information is needed to predict the rate of zebra mussel movement into inland waters, the types of habitats they will invade, and the impacts they will have on native mussels in these habitats.
- 6.2 Track the spread of zebra mussels and develop and maintain a GIS system to monitor their spread relative to the location of native mussel populations. The spread of zebra mussels should be monitored and the data reported in a readily available format. The USGS's Southeastern Biological Science Center, Gainesville, Florida, currently tracks the spread of zebra mussels. That database should be reviewed to determine whether modifications are necessary to meet the needs of native mussel conservation and aquatic resource managers.
- 6.3 Develop guidelines and thresholds (triggers) to assist managers in determining when, which species, and how many individuals of a species should be brought into captivity or relocated when it is determined they are at risk from zebra mussel infestations (see Strategy 9.5). Zebra mussels have decimated native mussel populations in the Great Lakes, and this non-indigenous species is now infesting native mussel beds in the Inland Basin. The continued existence of rare large-river mussels is now threatened by this invasion. Guidance is needed on when, which species, and how many individuals of a species should be brought into captivity or relocated when it is determined they are at risk from zebra mussels. Information also will be needed on the relationship between zebra mussel infestation rates and the survival of native mussels. It should be determined if portions, or an entire population, of rare mussels need to be rescued before infestation is observed or if native mussels can survive relocation after some degree of infestation.

6.4 Move native mussel species at risk into hatchery facilities or to locations within their historic ranges where zebra mussel infestations will be inconsequential or unlikely (see strategies under Problem 9). It appears that the greatest threat to native mussels from zebra mussel infestations will occur in large rivers and in rivers with upstream reservoirs. Consideration should be given to moving species at risk of extinction into suitable refugia.

6.5 Develop protocols to ensure that zebra mussels are not inadvertently introduced into new waters when native mussels are relocated. Because of the dire threat posed by zebra mussels, some mussel species will be moved into hatchery facilities or to locations where zebra mussels do not exist. Protocols should be developed and complied with to ensure that zebra mussels are not incidentally introduced when relocating native mussels.

6.6 Determine how zebra mussels spread to new waters. Barge traffic has been the primary zebra mussel transport mechanism in large navigable rivers, and recreational boats are the likely vector into smaller rivers and lakes. Definitive information on the zebra mussel's mode of transport could be useful in developing control procedures.

6.7 Investigate the feasibility of controlling the spread of zebra mussels through technological means. Research on the physical, chemical, and biological control of zebra mussels is urgently needed. Biological control of zebra mussels may offer the best option for conserving native mussels. However, extreme care must be taken to ensure that zebra mussel control methods do not jeopardize native mussels.

6.8 Inform the public about the threat zebra mussels pose to native aquatic species and other resources (e.g., sport fisheries, water supply facilities, and power plants). Public support will be needed to stem the invasion of zebra mussels into other waters. The public should be informed of the economic and ecological threat posed by zebra mussels and provided with information as to what they can do to reduce the species' dispersal rate (see strategies under Problem 7). If the spread of zebra mussels can be slowed, increased opportunities will be available to develop native mussel protection strategies.

PROBLEM 7: There is a general lack of concern, awareness, and understanding by government agencies, legislators, academia, and the general public about the ecological and economic value of our native mussels, other aquatic resources, and the anthropogenic impacts that threaten their continued existence.

GOAL: Enhance public and government agency understanding and support for federal, state, local, tribal, and private programs that protect and enhance natural stream ecosystems for the benefit of freshwater mussels and other aquatic and aquatic-dependent resources.

STRATEGIES

Note: Outreach is critical to the success of this National Strategy, and it is especially important to the successful implementation of strategies listed under Problems 2 and 10.

- 7.1 Compile an annotated list of existing freshwater mussel-related outreach material. Considerable educational material relating to freshwater mussels and the value of protecting natural stream ecosystems already exists.

7.2 Identify target audiences, evaluate the need for outreach material for these audiences, develop appropriate media to strategically convey focused mussel conservation messages to specific audiences. Identify target groups that can assist with mussel conservation and those that could be, or perceive they could be, impacted by the program. Where needed, develop specific outreach material for these target groups.

7.2.1 Develop and implement an educational program that increases public awareness of the plight of mussels and the benefits of maintaining the ecological integrity of aquatic ecosystems. The future of our nation's freshwater mussel fauna and other aquatic and aquatic-dependent biota (e.g., nongame and sport fish, neotropical migrants, amphibians, reptiles, small mammals, and wetland-dependent plants) will depend on the degree of public support for aquatic ecosystem protection and recovery programs. However, the public generally places little value on aquatic species (with the exception of some game species). Many people perceive the conservation of mussels and other invertebrates as unnecessary and wasteful of government funds. The public should be provided information on the following: (1) the plight of freshwater mussels; (2) their aesthetic, commercial, scientific, and ecological value; (3) the benefits other aquatic resources derive from maintaining mussels as a component of natural stream ecosystems; and (4) what they can do to help in this recovery effort. With this information, the public will be better informed when judging the benefits and costs of preserving mussel resources.

7.2.2 Develop and implement an educational program that increases government agency awareness of the plight of mussels and the benefits of maintaining healthy, intact aquatic ecosystems. The support of natural resources agencies and other agencies with programs that impact aquatic resources is critical to a successful mussel conservation effort. In order for administrators and other employees of these agencies to consider mussels within their program, they must be provided information as to the many ecological and social values of maintaining the biological integrity of freshwater ecosystems.

7.2.3 Develop and implement an educational program that increases the awareness of nongovernment organizations about the plight of mussels and the benefits of maintaining healthy, intact aquatic ecosystems. Many nongovernment organizations (e.g., TNC, American Fisheries Society, American Sports Association, Izaak Walton League, Science Educators of America, American Rivers, Association of Southeastern Biologists, and various universities, zoos, and museums) actively support aquatic resource conservation. These organizations should be (1) kept informed of this conservation initiative; (2) provided with educational materials related to the goals, strategies, and progress of this effort; and (3) encouraged to join in this conservation program.

7.2.4 Develop and implement an educational program that increases awareness within the commercial mussel shell industry and among pearl producers about the plight of mussels and the benefits of working jointly to maintain healthy, intact aquatic ecosystems. The commercial mussel industry and pearl producers view the value of

mussel resources from a different perspective than most natural resource managers, and they sometimes disagree on management issues. However, the commercial mussel industry, mussel resource managers, and mussel researchers are all interested in conserving the benefits obtained from sustainable native mussel resources. Cooperative efforts should be pursued that benefit the industry and the mussel resource managers' ability to conserve mussel abundance and diversity. There will be times when the desires of the industry and those of resource managers will conflict, but both groups need to understand that significant benefits can be derived by working together on common issues. The industry can assist researchers and managers by providing field expertise, assistance, and historical knowledge, by soliciting funds and providing facilities for research, by conducting outreach to the general public, and through support and actions to prevent further habitat degradation. Mussel researchers and managers can help to preserve mussel habitat and community structure for commercially valuable species, provide technical assistance on mussel propagation and holding technology, and set and enforce size and harvest regulations that ensure a sustainable mussel harvest.

7.3 Identify and develop specific educational/informational material and mechanisms to assist field biologists with implementing this National Strategy. This includes items such as an annotated bibliography of existing freshwater mussel literature (see Strategy 1.1.4), a database on the historic and current distribution of mussels (see Strategies 4.4 and 4.5), and an effective information transfer system on current mussel research, management, and conservation issues (see Strategies 1.1.2 and 2.2). In addition, the following strategies should also be implemented:

7.3.1 Develop a mussel key. The only available comprehensive mussel key was produced by EPA in 1973 (Burch 1973). This key has been a valuable resource, especially to people new to the field. However, it does not cover all species, taxonomic revisions have occurred since 1973, and the key is difficult to use. A new or revised version of the existing mussel key, making ample use of color photographs, would help increase the identification abilities of new mussel workers.

7.3.2 Develop training courses and seminars on mussel identification, basic biology, culture techniques, sampling methods, and habitat restoration/protection. As more individuals and agencies become involved in this mussel conservation effort, training sessions and seminars will be useful.

PROBLEM 8: The survival and recovery of many mussel species will require the development of artificial propagation and juvenile mussel reintroduction techniques, but these methods have not been perfected.

GOAL: Develop, evaluate, and use the technology necessary to propagate and reintroduce juvenile mussels on a large scale.

STRATEGIES

8.1 Develop glochidia transformation technology for native mussels. Artificially propagated juvenile mussels are needed for

four primary purposes: to (1) augment populations when population size of a rare species is too small, young, or old to support reproduction; (2) establish new populations when the translocation of adults is not possible; (3) maintain a captive population when the species' natural habitat is deemed unsuitable; and (4) for bioassay research. Once developed, the propagation technology must be adapted to larger-scale operations in order to produce sufficient young mussels for these activities.

- 8.1.1 Perfect an artificial culture medium. Juvenile mussels have been propagated using artificial media, but results have not been consistent with all species. Additional research is needed to improve applicability and usefulness of this technique.
- 8.1.2 Perfect artificial culture using host fish. The use of fish hosts for glochidia transformation has been developed and is used by many researchers. However, because of fish host specificity and the need to maintain large numbers of a variety of fish species, this method is labor intensive. Suppression of host fish immunity, identification of alternative host species, and the use of non-indigenous fish should be evaluated to determine if healthy juvenile mussels can be produced.
- 8.1.3 Determine the feasibility of propagating and rearing juvenile mussels in hatchery raceways, ponds, or tanks. Other propagation techniques currently under development include (1) artificially infesting fish with glochidia and releasing the fish into a hatchery raceway, and (2) holding gravid adult mussels in a raceway with their fish hosts and allowing the fish to be infested naturally. In both cases the juvenile mussels can drop off the fish, be reared in a raceway, pond, or tank and be removed when they are large enough for stocking. If successful, these methods would require less manpower than other artificial propagation techniques that are under development.
- 8.2 Develop diets for artificially propagated juvenile mussels. Once juvenile mussels are produced, they must be fed and reared to a size suitable for release. The technology to feed juvenile mussels is not fully developed, and has been tested on only a few species. The food and feeding regimes must also be adapted to large-scale operations in order to make propagation a feasible management tool.
- 8.3 Determine the viability of artificially propagated juveniles. The survival and growth of medium-produced and artificially reared juveniles should be compared to those of naturally produced juveniles to evaluate their suitability for release in restoration and recovery programs.
- 8.4 Conduct a comprehensive review of foreign and related literature that could have application in mussel propagation research. As Asian countries have a wealth of experience in freshwater mussel culture, their literature should be translated so techniques can be tested and implemented here.
- 8.5 Conduct a review of mussel culture activities outside the United States (e.g., China, Japan, Australia, and Indonesia) and organize an international symposium on artificial propagation. Foreign researchers are working on propagation technology. An international symposium on the subject would bring these various groups together to share research results and explore new research directions.
- 8.6 Identify criteria for selecting federal, state, tribal, and private

hatchery facilities that could be used for large-scale mussel propagation. Although propagation technology is not fully developed, existing hatchery facilities will eventually be needed to produce juveniles for reintroduction. If the facility managers know that they might be requested to propagate mussels, they could consider these criteria when planning modifications at their facility. (Appendix III contains draft criteria modified from the Service's facility criteria.)

- 8.7 Determine the risks associated with mussels, their fish hosts, and associated diseases escaping from the facility into non-historic habitat. Whenever species are moved into areas outside their historic range there is always a risk that they will escape and become established. If mussels and their associated fish hosts are to be propagated and held outside their historic range, an assessment should be made of the risk of escape and potential consequences. The Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish (available on the Internet at <http://www.nbiap.vt.edu>) will be considered in the course of propagation and containment activities. Mussel containment activities will not continue where the consequences of escape are likely and severe.
- 8.8 Develop the technology to reintroduce juvenile mussels into historic habitat. The reintroduction/relocation of adult mussels has met with limited success, and the feasibility for releasing juvenile mussels into the wild and artificially infesting and releasing fish hosts needs to be tested. Additional research on such factors as habitat suitability, size and number of juveniles to release, method of release, and time of release are needed.
- 8.9 Identify streams for reintroduction and augmentation of mussel populations. Federal and state natural resources agencies should form partnerships to develop a prioritized list of streams that can be used for reintroduction and augmentation of mussel populations.

PROBLEM 9: The survival of rare mussels will require the ability to hold them in captivity or in refugia and to translocate adult mussels to reestablish populations. However, these techniques are not adequately developed for implementation by resource managers.

GOAL: Develop, evaluate, and use the techniques necessary to hold and translocate large numbers of adult mussels.

STRATEGIES

- 9.1 Develop protocols to relocate adult mussels. Adult mussels are generally relocated for two reasons: to (1) remove them from an area when a development project or other factors threaten their survival and (2) release them back into restored historic habitat. Efforts to relocate adult mussels have met with varied success, nevertheless, this tool is essential to mussel conservation. For example, zebra mussels are currently threatening rare mussel in the Ohio and Mississippi River systems (see Problem 6). To save some of these native species, it will be necessary to move some rare and commercially valuable species to areas that will not be threatened by the zebra mussel (see Strategies 6.3 and 6.4). Also, adult mussels can be relocated in order to reestablish extirpated populations when sufficient specimens are available in a donor population.
- 9.2 Develop criteria for mussel relocation. Develop a checklist of the physical, chemical, and biological parameters (e.g., habitat type, pH, oxygen requirement, and number of individuals

needed for a self-sustaining population) to be considered before attempting to translocate mussels or hold them in refugia. This guidance should address moving species between watersheds and introductions into nonhistoric habitat. The guidance should also stress the need to monitor and fully report project results.

- 9.3 Develop mechanisms for the long-term monitoring of transplanted mussels. Once released into the wild, individual mussels are difficult to relocate, complicating the assessment of release success. For example, research was conducted in the early 1980s on the feasibility of tagging mussels with magnets and radio tags, but did not provide any reliable technology. Since radio telemetry has improved in recent years, another mussel tagging study may be warranted. Other avenues of relocating and monitoring transplanted mussels should be investigated as well.
- 9.4 Develop technology to maintain adult mussels in captivity. Many species are so rare or so threatened by habitat destruction or other factors that they are likely to become extinct in the wild in the foreseeable future. As mussels are long-lived, it may be possible to maintain some species in captivity for extended periods. When habitat is restored or suitable habitat is located, these individuals or their propagated offspring could be returned to the wild. The technology for the long-term maintenance of captive mussel populations is not fully developed. Research on the feeding and habitat requirements of captive-held adults is crucial.
- 9.5 Develop guidelines with thresholds (triggers) to assist managers in determining when individuals of a mussel species should be brought into captivity. Many factors threaten the continued existence of native mussels. Guidelines are needed to assist managers in determining when a species is so threatened by these factors that it should be brought into captivity or relocated to a more secure location (see Strategy 6.3).
- 9.6 Develop criteria for selecting an appropriate facility to be used for captive mussel holding and identify specific facilities that could be used in this effort. These criteria will assist managers in determining if their facilities are suitable for captive holding. If the facility managers know they might be requested to hold mussels, they could consider these criteria when planning modifications to their facilities. Secure appropriate commitments from agencies or organizations for facility space in areas where there is an imminent need for captive holding (see Strategy 8.6).
- 9.7 Determine risks associated with species escaping from the facility into nonhistoric habitat. (See narrative under Strategy 8.7).
- 9.8 Develop a health strategy for captive mussel populations. This would include the development of techniques for disease diagnosis, determination of disease vectors, and disease control. If adult mussels are to be brought into active fish hatchery facilities, the effects of mussel diseases on fish and fish diseases on mussels should be assessed.
- 9.9 Develop mussel cryopreservation technology. Cryogenic preservation could maintain mussel genetic material (much like seed banks for endangered plants) until such time that the habitat is suitable for reestablishing the species. Additionally, if a mussel population were lost to a catastrophic event, such as a toxic chemical spill, cryogenic preservation could allow for the eventual reestablishment of that population using preserved genetic material. As cryopreservation techniques for

other faunal groups are developed, the technology should be adapted and tested on native mussels.

PROBLEM 10: Current funding levels are not sufficient to address identified information needs or to implement this National Strategy.

The outreach strategies identified under Problem 7 are critical to the success of the following strategies.

GOAL: Increase available funding levels and develop other means to increase mussel conservation efforts.

STRATEGIES

- 10.1 Develop partnerships and seek funding from government agencies, private organizations, foundations, industries, and individuals. No one agency or organization has sufficient funds or expertise to conserve and recover our nation's mussel fauna. Partnerships, cooperative ventures, and funding from within and outside government are essential to program success. Additionally, mussel conservation will not succeed unless it is integrated with other aquatic ecosystem conservation efforts. The benefits of mussel conservation must be linked to other aquatic resource benefits.
 - 10.1.1 Seek funding assistance from federal, state, and tribal agencies that have direct involvement with aquatic resources management. Many natural resources agencies, such as the Service, BRD, USGS, Corps, TVA, EPA, USFS, NPS, and state and tribal natural resources agencies, are already funding projects directly related to mussel conservation. Develop specific proposals and solicit their help in the conservation effort. These agencies should be encouraged to examine their existing authorities to determine how they could expand into mussel conservation.
 - 10.1.2 Seek funding for mussel conservation from agencies or organizations that have activities which impact mussel communities. Many regulatory agencies oversee programs that secondarily benefit mussels; they might be willing to strengthen their programs to improve the protection of mussel resources. Pursue cooperative funding that satisfies an agency's needs and promotes mussel conservation. Consider establishing mitigation trust funds to help compensate for the loss of mussel resources caused by development projects. A trust fund was established to mitigate for the loss of a mussel bed on the Ohio River. This trust now provides funds for mussel conservation projects that benefit Ohio River mussels (Marshall et al., 1993).
 - 10.1.3 Evaluate funding alternatives, such as a tax on exported shells, commercial mussel harvest fees, or a tax on the import of products made from native shells. Some states already impose a tax on harvested shells, and the funds are used for mussel conservation efforts. A federal tax on domestic shell exports or the foreign import of mussel-derived products should be considered.
 - 10.1.4 Seek funding assistance from non-government agencies and organizations, businesses, and foundations. Many organizations fund conservation projects or provide in-kind support. If one organization provides funding, other organizations are often more willing to match the original funds. Solicit the support of such

organizations and build cooperative efforts among these groups.

This National Strategy presents an outline of suggested goals and strategies for a national mussel conservation program. These strategies do not encompass all the conservation activities that are currently under way nor do they identify all activities that will be needed for the long-term conservation of mussels. They are offered as guidance to provide a national

mussel conservation perspective and to help various organizations identify the types of conservation tasks that could be implemented to assist in the greater conservation effort. Furthermore, the National Strategy is intended to be a dynamic document that will be revised periodically as new information becomes available and new strategies are developed. The authors welcome any comments and suggestions that would help enhance short- and long-term mussel conservation goals.

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Appendix I

Participants at the April 1995, mussel meeting in Roanoke, Virginia, and other individuals who were involved in drafting the document, or provided written comments.

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Appendix II

Criteria for Prioritizing Projects and Activities Identified in this National Strategy

- Project has partners or has the potential to create partnerships.
- Project work is related to priorities for geographical distribution and species diversity and considers the degree of threat.
- Project addresses both the status and trends of the mussel populations.
- Project focuses on the aquatic system within the watershed (USGS quadrants).
- Project fulfills the objectives of existing management or recovery plans.
- The project is feasible and achievable (i.e., cost-effective; uses the best choice of methods).
- Project does not unnecessarily duplicate existing studies.
- Project provides a short-term solution until a long-term plan is developed, or itself offers a long-term solution.
- Project has a monitoring component.
- Projects that prevent extinction are of a higher priority than projects that maintain existing populations, and maintenance projects are of a higher priority than recovery projects.
- All other options have been explored (e.g., habitat restoration or translocation).
- Applicability of research to multiple species (national research receives a higher priority than local research).

Appendix III

U.S. Fish and Wildlife Service Facility Criteria

- The activity must be considered essential and must be justified in management, conservation, or recovery plans, etc. For Service facilities, the work needs to meet the goals of the ecosystem plan.
- The water quality and supply must be known and must be compatible with species to be held.
- Facilities should ONLY work with mussels from the same basin or watershed. Mussels will only be allowed to be cultured or held outside of their native basin or watershed under a special permit or in cases of emergency (i.e., the threat of extinction) and with approval from state natural resources agencies and the support of the conservation community. Culture of non-native mussels shall go forward only in well-confined facilities with detailed operations management plans.
- Facilities must have a water source free of zebra mussels.
- Facilities must have a wet lab or have ready access to one.
- If the facility staff DOES NOT have the technical expertise required, the facility's project leader must procure the necessary training or secure the necessary expertise through cooperative arrangements with appropriate experts.
- Facilities must have an available and suitable food source. If a natural source is not available, a facility must have the space and expertise needed to produce food.
- Projects should be cooperative ventures involving federal, state, tribal, or private organizations. For example, projects designed by the Service should involve hatcheries, Fish Health Labs, and Fish Technology Centers. If federally listed species are concerned, involve Ecological Services.
- The project must comply with all federal and state permit requirements.
- The introduction of mussels into a facility should not significantly affect the existing fish production program.
- A contingency plan should be prepared that addresses how listed mussels will be rescued in case of flooding or other disaster.

Note: Criteria should be a function of the project objectives (e.g., refugia, propagation, and research). If particular criteria are not applicable to the project, compliance with that particular criterion is not necessary. If this is the case, justification for not complying with the criterion must accompany a proposal. For example, the need for raceways or ponds is dependent on the species and project.

Proceedings of a Workshop on
SPATIAL DATA AND REMOTE SENSING
IN
INVERTEBRATE FISHERIES

Held in Conjunction with the Annual Meeting of the
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89th Annual Meeting

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GUEST EDITOR
Gary Smith

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PREFACE

GARY SMITH

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This 2-day workshop was inspired by the former director of the Cooperative Oxford Laboratory, Aaron Rosenfield. His interest and enthusiasm in the benthic remote sensing work done by the Mapping and Analysis Project (COLMAP) at the Laboratory led him to propose a gathering of other individuals and groups involved in related work. His concept led those of COLMAP along a vague path as to what a workshop on aquatic remote sensing of invertebrate species was to include or accomplish. With the generous offer of the National Shellfisheries Association to allow us to hold the workshop at, and just prior to, their annual meeting, we had a place and fellowship. With generous financial assistance from the Corps of Engineers and the National Environmental Satellite Data and Information Service, we had a gathering and generated this publication.

On short notice, we solicited abstracts relating to the subject. Our guidelines were that emphasis was to be placed on strong visual presentations. An early concern was that abstract response would be poor. Ultimately, we were delighted with the response. We selected 21 presentations, cramming our 2-day time slot. Abstracts seemed to fall into four principal categories: Satellite and Atmospheric Remote Sensing, Geographic Information System (GIS) Data Analysis, In-water Remote Sensing, and Management. Lead off speakers graciously introduced and monitored each of the topical areas. After leaving the workshop, presenters and audience felt that the workshop concept was strong and expressed interest that a similar workshop be held later.

As things go, it has taken some time to assemble and review the extended abstracts we solicited for publication. The National Shellfisheries Association graciously offered to allow us publication within its journal. We finalized with 15 papers. To assist in augmenting the theme of our workshop, we requested that authors submit additional, noncited, references pertaining to their general topical area. These we have included. Submitted abstracts organizationally fell within the topical areas of publication order listed below. An original concern was with standardizing the format for each of the submitted abstracts. Realizing that this would involve much rewrite and possible reinterpretation of the authors' intent, we left the abstract organization alone.

In our initial presentation guidelines, we stressed the importance of visuals (including color) to the workshop. We gladly accepted a large number of figures with the extended abstracts. In some cases, however, if a color figure could be reproduced as black and white without loss of interpretation, we did so to reduce costs.

Contents

Overview and Management

Brown

Satellite Remote Sensing

Perry

Tester

Ladner

In-water Remote Sensing

Cutter

Mayer

Tracy

Caddell

Service

Zhou

Berk

Geographic Information System Data Integration

Rubec

White

Parker

Smith

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SHELLFISH BENTHIC HABITAT ASSESSMENT IN THE CHESAPEAKE BAY: PROGRESS TOWARD INTEGRATED TECHNOLOGIES FOR MAPPING AND ANALYSIS

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ABSTRACT Combinations of mechanical and remotely sensed technologies were applied to the task of developing techniques to assess and chart the physical condition of oyster habitat in the mesohaline Maryland Chesapeake Bay. Acoustic sub-bottom profiling equipment, side-scan sonar, underwater video, sediment analysis, and seafloor classification systems were tested for their capability in characterizing important components or conditions of the benthic environment, as well as their ability to integrate and display this information via linked Global Positioning System (GPS) and Geographical Information System format (GIS). Acoustic seafloor classification fully integrated with shipboard and land-based GIS, linked to bottom validation techniques, seems to offer the most effective solution to oyster habitat assessment at virtually any scale of interest.

KEY WORDS: Geographical Information System, shellfish, hydroacoustics, seafloor classification

The chronic and extreme long-term decline of oyster harvest in the Chesapeake Bay has been blamed on a combination of three factors: overharvest, disease, and loss of oyster habitat. Of the three factors, the chronic effects of habitat loss through destruction of reef structure and sedimentation may be the most difficult to quantify on a large scale over time. In the soft bottom environment of the Chesapeake Bay, live or dead oyster shells protruding above the sediment (cultch) are the principal available substrate for spat settlement and growth. Field assessment methods, coupled with precise Global Positioning System (GPS) navigation were integrated with Geographic Information System (GIS) data management and analysis to begin the task of examining which suite of technologies could be best employed to characterize the condition of cultch habitat.

Three aspects of information on the Chesapeake cultch resource are desired: (1) to identify a level of sedimentation on cultch that could potentially degrade recruitment; (2) to assess the density and geographic extent of exposed cultch on the bottom rapidly and accurately; and (3) to identify buried deposits of oyster shell indicative of historic reefs and potentially available for reclamation. If such were practical and efficient on a large scale, long-term monitoring of the cultch resource as well as determination on the effectiveness of restoration technologies and harvest practices upon the oyster resource could be realized.

Combinations of mechanical grabs, acoustic sub-bottom profiling equipment, side-scan sonar, underwater video, and seafloor classification systems were applied to the task of assessing which suite of technologies best meet the requirements of the objectives. Application of remote acoustic technologies required the integration of ground truth data to allow for calibration and classification. A principal difficulty encountered in the Chesapeake Bay is an almost complete lack of visibility throughout most of the year for visual or video ground truthing. Various scales of resolution also complicate the analysis. The importance of detecting light sedimentation requires a millimeter level scale; whereas, buried cultch detection is on a scale of meters.

A primary aspect of this work was to determine if baseline data depicting the historic extent and condition of the oyster "reef" was, indeed, a valid baseline depiction of the extent of the cultch resource. Baseline data were from two principal sources. The Yates

Survey (Yates 1911) was an extensive and detailed charting of turn-of-the-century Maryland oyster bottom. Acreages calculated as laying within the polygons depicting individual oyster bars have often been used as a baseline acreage of oyster habitat for Maryland. The Maryland Bay Bottom Survey (unpublished data) charted cultch content largely by the use of a microphone dragged along the bottom. A reduction in cultch acreages between the two surveys has been used as evidence of widespread areal loss of reef (Rothschild et al. 1994).

Preliminary field assessment was to determine sub-bottom characteristics on and transitional to historic oyster bars. "Chirp" sonar (Schock et al. 1989) manufactured by *Edgetech*, *DataSonics*, and a unique sub-bottom classification system assembled by the Naval Research Laboratory (Lambert et al. 1993) were tested in and around the mesohaline Choptank River system (Figs. 1, 2). Interpretation of results was often difficult because of the presence of highly varying patterns and quantities of methane gas within the sediment that effectively masked any sub-bottom features in "soft" portions of the bottom. Buried "hard" terraces were, however, exactly matched with some original boundaries of charted oyster bars (Fig. 3). Where these terraces (or portions of terraces) reached the surface, cultch was regularly found to be a principal surface component. Piston coring of one of these buried terrace slopes found oyster shell fragments at a depth corresponding to the slope depth indicated on the sub-bottom trace.

Interpretation of sub-bottom traces (although often intuitive), additionally showed high correlation with bottom classifications of the Maryland Bay Bottom Survey. Sub-bottom profiling also revealed that boundaries of many of the original charted Yates Bars of Maryland often contained large sections of muddy or sandy bottom with no supporting substrate at depth. These areas were clearly never capable of supporting an oyster community. Yates polygon oyster bar boundaries were often seen in many cases as enclosures of several isolated and irregular patches of "terraces" identified by sub-bottom profiling. The Bay Bottom survey commonly was able to identify such areas as exposed cultch. It became apparent that utilization of Yates survey acreage as a turn-of-the-century baseline habitat index may bias true turn-of-the-century oyster habitat in a positive fashion.

Sub-bottom profiling information provided limited and highly

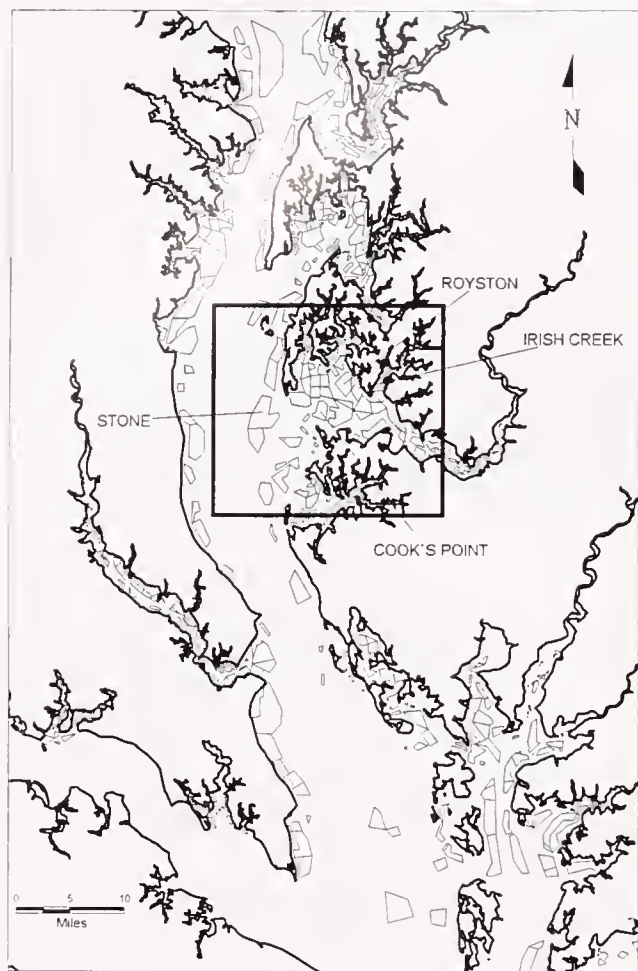


Figure 1. Study area in the region of the Choptank River on the eastern shore of the Maryland portion of the Chesapeake Bay. Charted oyster bar boundaries at the turn of the century (Yates 1911) are shown in light line. Oyster bars referenced in this paper are identified.



Figure 2. Enlargement of the mouth of the Choptank River study area. Yates oyster bar perimeters (circa 1911), Stone Rock, Cook's Point, and Royston Bar, are highlighted.

intuitive information about density and sedimentation level of exposed cultch. Difficulties also arose in how to integrate interpretive and visual output within a GIS-based system. At this point, feature files, indicating points of interest along transects, keyed to computer-produced images were the only feasible way for us to link visual output to the GIS.

Presently, we are utilizing two techniques for bottom calibration for remote systems: video and ponar dredge samples. Traditional mechanical bottom sampling devices may eliminate evidence of light sedimentation over cultch during their deployment and retrieval. From such grab samples, identification of the degree to which oyster shell is buried is often dependent upon burial to an anaerobic depth producing "blackened" shell. Although initial video results produced from a bottom camera were often of extremely poor quality because of poor water clarity, preliminary video along sub-bottom profile tracks identified the high degree of sedimentation on shell in traditional oyster regions in the mesohaline portion of Chesapeake Bay. High sediment loads on recent oyster shell repletion plantings was also observed. Video images and classifications are being linked to GPS location along sub-bottom transects via the GIS to allow for retrieval (Fig. 4).

Side-scan sonar was employed to determine its potential in characterizing the presence of various densities of cultch on the

bottom. It was hoped that results would allow for wide swaths of the bottom on either side of the survey boat to be classified and presented in two-dimensional GIS representations. Success was mixed. Traditional wide-swath equipment commonly employed in deepwater surveys produced very limited discernment of bottom type in our shallow waters. Ground truthing was always necessary to identify the cause of reflectivity differences. Rapidly changing depth, water mass variation, and a regular need to adjust signal and reception parameters all limited detail for purposes of mapping.

Utilization of high-resolution, narrow-range side-scan equipment (*Sea Scan PC*) greatly increased surface resolution. Survey width was, however, reduced from approximately 100 m on each side of the vessel to a maximum of 40 m. An important tool in side-scan integration into a GIS format is computer mosaic technique. This procedure electronically assembles the individual survey swaths of data over the bottom into a two-dimensional surface that can be draped over other data layers generated by the GIS (Fig. 5). Although we are involved in developments in this field, this approach may be costly and survey intensive for purposes of wide-scale habitat assessment. Additional problems involve the storage and processing of large quantities of data generated by such surveys.

Recent advances in high-resolution laser line scan capability

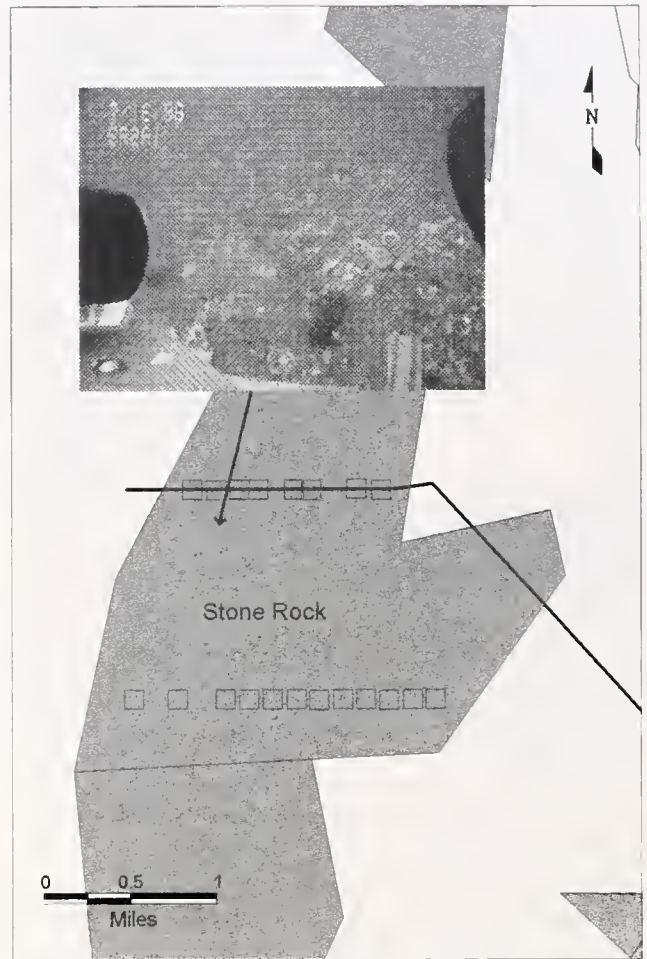


Figure 4. Video ground truthing of survey tracks on Stone Rock oyster bar. The black line indicates survey track and small boxes, video calibration sites. Geographical Information System image integration allows for spatial capture and simple retrieval of image data.

application of ground truthing instead allows for the compartmentalization of returning echoes into characteristic bottom types.

Two available Seafloor Classification Systems on the market employ two different approaches to echo analysis. One system requires *a priori* knowledge of principal bottom types in the region of interest, and the other does not. *Roxanne* uses components of the first and second returns of the transmitted sound pulse to characterize the bottom in a two-dimensional axis. These axes have a quasirelationship to harness and roughness. Various combinations of these two (E-values) can be assigned to different types of bottom substrate (i.e., bedrock, cobbles, sand) (Dyer et al. 1997).

Another system, *QTC* requires *a priori* training of the system to allow particular waveforms of the returning echo to be assigned to predetermined bottom types held in memory. In such analysis, the confidence, or level of association can be assigned to each returning echo.

Both systems were employed over sections of the bottom exhibiting highly variable composition. Although comparative analysis is not yet complete, it is apparent that both systems can discriminate fine variation in bottom character. Determining precisely what acoustic properties cause this discrimination is still, however, a key requirement of ground truthing. Continued research in trans-



Figure 5. A side-scan sonar mosaic draped over a bathymetric mesh of bottom depth within Cook's Point oyster bar. The dark black line to the left is the western oyster bar edge as delineated by Yates (circa 1911). The side-scan image has been electronically draped over the bottom contours. The survey path was horizontal through the middle of the image. The field of view to either side is approximately 100 m. With proper equipment, multiple passes over the bottom could be electronically linked into a large two-dimensional image. In shallow water estuarine conditions, Side Scan Sonar data integration with Geographic Information Systems is subject to many forms of operational and practical difficulties. In addition, extensive field validation is required to determine the cause of differences in acoustic reflectivity.

ducer design, frequency variation, and classification analysis may continually improve system effectiveness.

Seafloor Classification Systems may have high potential for estuarine habitat assessment, because its technology mates well with GIS/GPS data integration. Data are displayed in real time, allowing survey adjustments in the field. Data can be reduced to simple vectors, with time and location GPS stamps. Survey track data are amenable to a wide range of GIS incorporations (Fig. 6). Our principal display mode is transformation to a grid cell data model (Fig. 7). Cell size can be adjusted to the survey requirements. Interpolation techniques allow for the generation of continuous surface renditions of the survey area (Fig. 8).

Confidence intervals may be generated for commercial Seafloor Classification Systems. This statistic provides an indication as to how well system software matches a returning echo to a stored library echo. Similar statistics could be developed to assess high variation between signal returns along a section of boat track. Both situations call attention to potential bottom types that are unknown or areas where bottom transition is occurring.

Future applications for Seafloor Classification Systems will most likely call for closer integration with GIS. Such linkage will allow for fuller multifacet analysis and display of collected data. A current drawback of these classification systems is that any change in system components or settings may result in entirely different

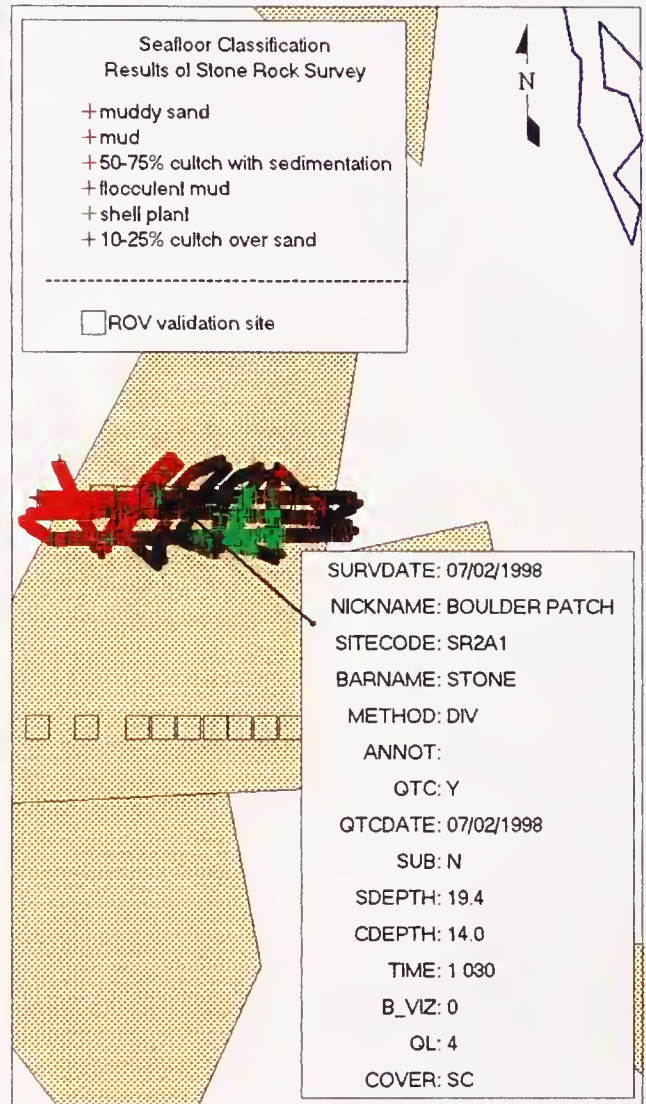


Figure 6. An example of a Seafloor Classification System (SCS) survey linked to Geographic Information System (GIS) display. Individual SCS point data are easily integrated into GIS storage format for display with other data files. In this figure, diver validation data are linked to the survey location.

classifications. Integration of GIS may again assist in calibrating surveys over time.

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Marine Sonics Technology Ltd., White Marsh, Virginia, "Sea Scan PC";
Questar Tangent Corporation, Marine Technology Centre, Sidney, BC, Canada, "QTC View."

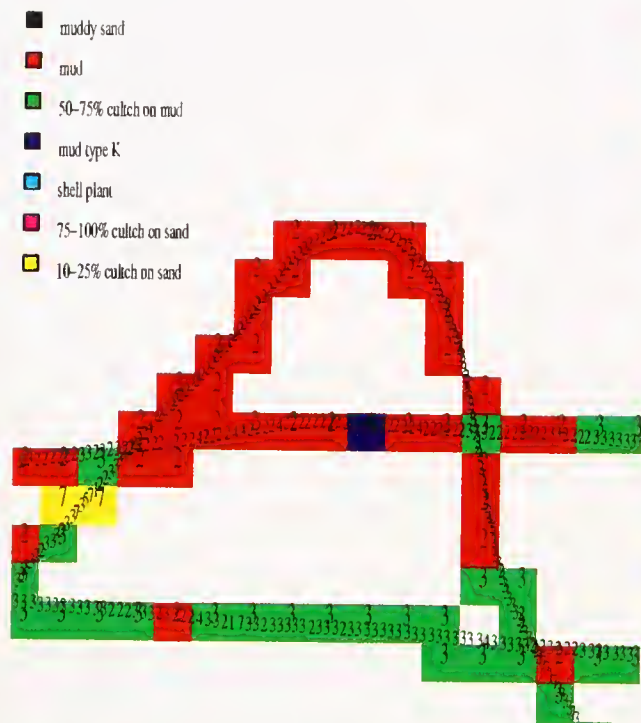


Figure 7. SCS survey data transformation to a grid cell model. Individual categorical bottom characterizations are shown along three survey tracks. Interpolation routines assign the most prevalent category within a chosen grid cell size to that cell. Cell color indicates bottom category type.

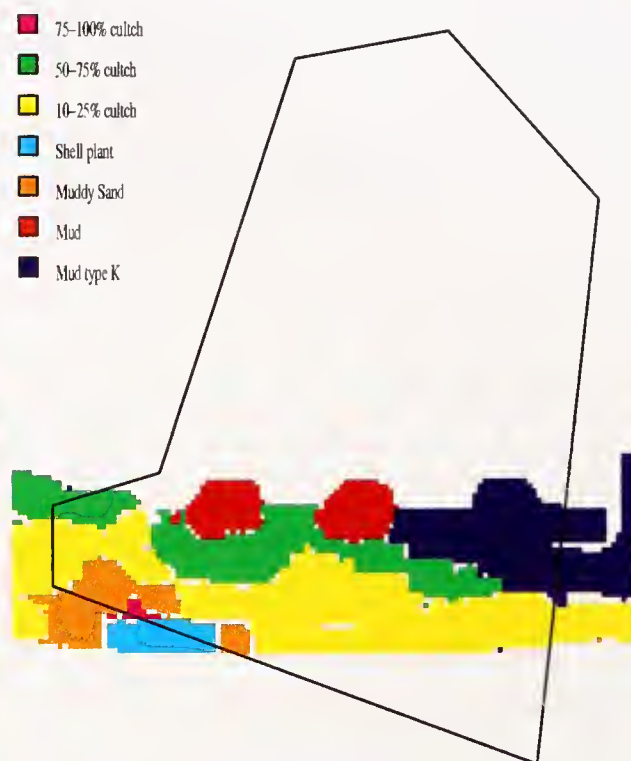


Figure 8. Continuous surface interpolation of SCS data. Data such as those generated for Figure 7 were used to demonstrate habitat type on lower Cook's Point oyster bar. Note small grid cell size utilized.

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APPLICATION OF REMOTE SENSING TO SETTLEMENT OF *CALLINECTES SAPIDUS* MEGALOPAE IN THE MISSISSIPPI BIGHT

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ABSTRACT Blue crab larvae undergo zoeal and megalopal development in offshore waters of the Gulf of Mexico. The surface-residing planktonic larvae are dependent upon advective currents to return them from sea at the appropriate time for settlement in near-shore habitats. Settlement is episodic. The periodicity of settlement is similar over years; however, magnitude is highly variable. A numerical model of the gulf was used to determine advective pathways and environmental forcing conditions affecting successful returns. Two factors were found to be important: wind stress and timing of the break-off of Loop Current eddies. Interannual variation in wind stress was most significant.

KEY WORDS: Blue crab, Gulf of Mexico, currents, wind stress

Fisheries for the blue crab, *Callinectes sapidus*, are characterized by annual, seasonal, and geographic variations in harvest. The causes for these variations remain poorly understood, with resulting uncertainties for fisheries management. Although many factors influence population size and a particular year-class strength, population levels are initially affected by recruitment dynamics. Because blue crab recruitment processes seem to be physically mediated and involve large-scale environmental features, the application of remote sensing technology can be especially useful in examining the influence of oceanographic and meteorological events on larval recruitment to a particular population.

Callinectes sapidus larvae are exported from northern Gulf of Mexico estuaries, with growth and development through the seven zoeal stages and metamorphosis to the megalopal stage occurring on the continental shelf (Perry and Stuck 1982, Truesdale and

Andrzejak 1983). Development through the larval stages usually requires from 30 to 50 days. Megalopae possess behavioral adaptations that maintain them in surface waters (Sulkin and Van Heukelem 1986) where the advective currents, upon which they are dependent for transport, return them from the sea at the appropriate time for settlement in near-shore, shallow water habitats. Megalopal settlement in northern Gulf estuaries is episodic (Perry *et al.* 1995, Rabalais *et al.* 1995). Temporal periodicity of settlement events is similar from year to year; however, the magnitude of settlement is highly variable (Figs. 1, 2). Extremes in settlement occurred in 1991 (high) and 1996 (low). Shelf circulation patterns and meteorological conditions (wind stress) were different in these 2 years, suggesting a causal relationship may exist between these parameters and settlement in the Mississippi Bight (Figs. 3, 4).

The Gulf of Mexico is a complex system with unique physi-

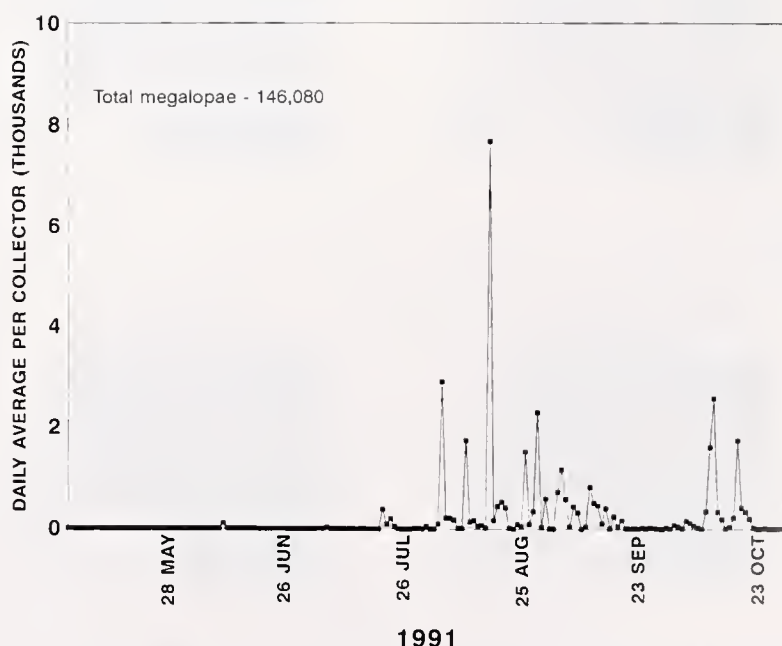


Figure 1. Daily average number of megalopae per collector, 1991; dates represent full moon.

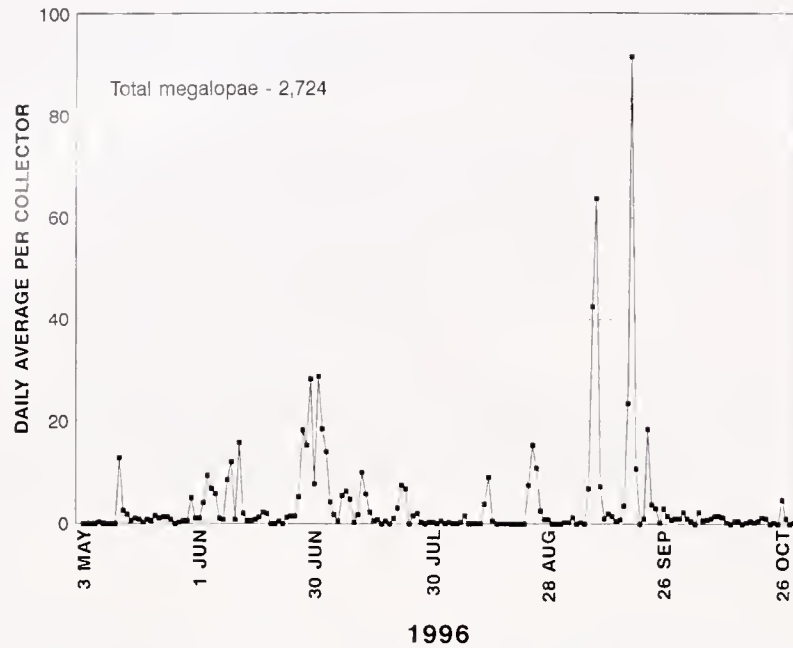


Figure 2. Daily average number of megalopae per collector, 1996; dates represent full moon.

ographic and hydrographic features. The Loop Current is the dominant hydrologic feature in the Gulf. The current is known to shed cyclonic (Elliott 1979, Vukovich and Maul 1985) and anticyclonic eddies (Cochrane 1972, Elliott 1982) that affect circulation patterns in the eastern and western Gulf, respectively. The Mississippi

River also exerts considerable influence on circulation over the continental shelf and beyond. Approximately 51% of the riverine discharge for the contiguous United States flows into the Gulf, with 73% of this discharge carried by the Mississippi River (Ward 1980). Coastal climatology of the northern Gulf is largely dictated

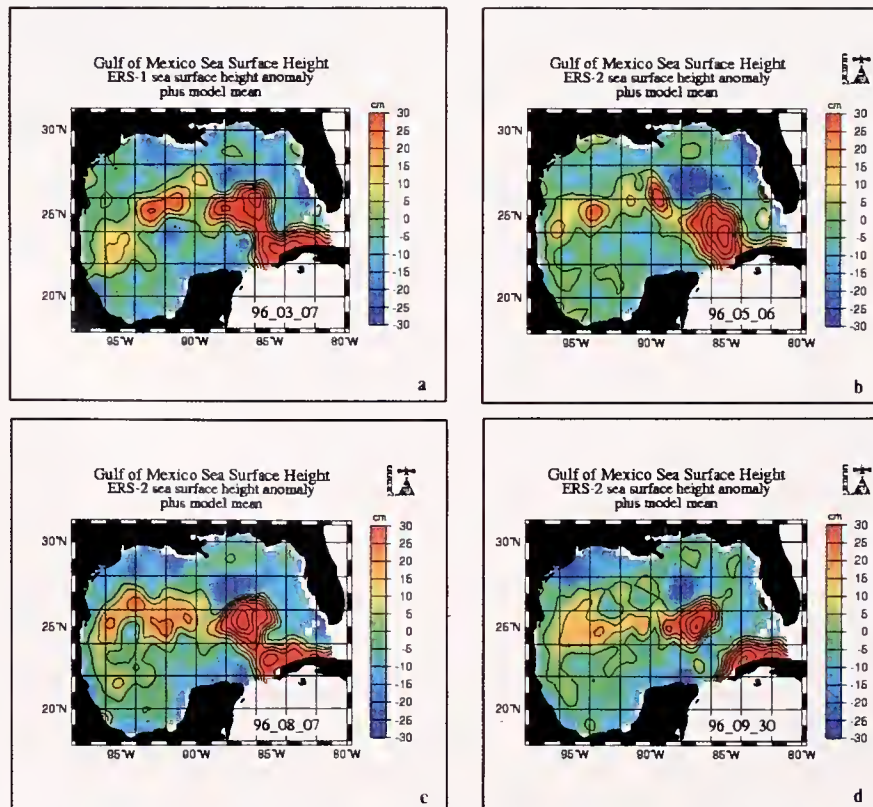


Figure 3. Altimetry maps of sea surface height in the Gulf of Mexico in: (a) March of 1996, (b) May of 1996, (c) August of 1996, and (d) September of 1996.

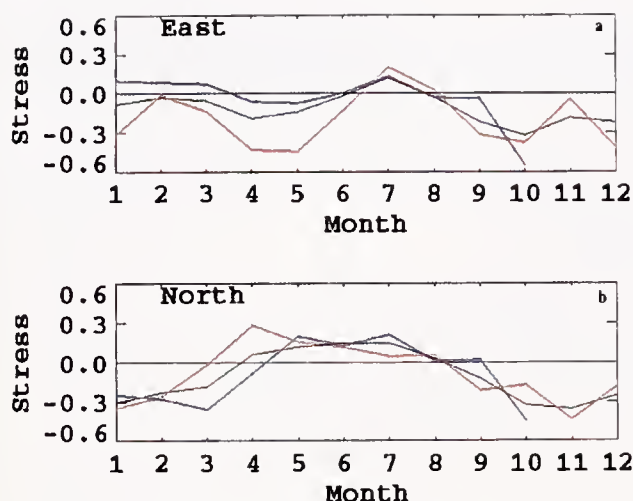


Figure 4. Winds from NDBC buoy showing eastward (a) and northward (b) wind stress in 1991 (red) and 1996 (blue) and the average from 1991 to 1996 (black).

by the subtropical Azores–Bermuda High (Eleuterius and Beaugez 1979, Ward 1980). Onshore winds are most prevalent in the summer when the high is strongest and farthest northwest. As the high weakens in early fall, coastal areas become subject to the weather systems of the midlatitude westerlies. Gulf tides, which co-oscillate with those of the North Atlantic (Zetler and Hansen 1972), are of small amplitude relative to tides along the Atlantic and Pacific coasts. Small amplitude tides in conjunction with a broad shallow shelf in the northern and eastern Gulf contribute to meteorological “forcing” of coastal processes (Smith 1977, Chuang *et al.* 1982, Schroeder and Wiseman 1986, Schroeder *et al.* 1987).

During 1996, the Loop Current shed eddies in the spring and summer. Eddy shedding is a repetitive event that commonly occurs at 8 to 10 month intervals. These shedding events occur via instability processes (Hurlburt and Thompson 1980) and, thus, are not amenable to prediction. The occurrence of an eddy shedding event during the spring and summer is not statistically unusual. However, it is, indeed, unusual to have two events occur in such a relatively short time span and cover the biologically important spring and summer seasons. The sea surface height (SSH) maps shown in Figure 3 were created using ERS-1/2 altimetry and a numerical model of the Gulf of Mexico. The model-determined mean sea level was added to the satellite-determined SSH anomalies to recover real sea surface heights. The Loop Current is clearly seen in the high SSH intrusions through the Yucatan Straits, and the Loop Current eddies are seen as closed high SSH to the north and west of the straits. In these four maps, the two eddies shed from the Loop Current can be readily seen. The first occurred

between March and May and the second between August and September 1996. In March, the Loop Current is seen to be highly extended into the northern Gulf of Mexico. A previously shed eddy can also be observed in the western Gulf. By the beginning of May, a recently shed eddy can be observed to the northwest of the Loop. Although small, this eddy is shed relatively far toward the north, where its impact on the Mississippi Bight can be important. In a normal eddy-shedding event, the newly formed eddy takes much more of the Loop water with it, and the reattached Loop is subsequently found far to the south. In this event, the eddy was relatively small, and the Loop continued to extend to the north for its second shedding event. By early August, the Loop Current is clearly in the process of pinching off again, and by the end of September a larger eddy has been separated. From a numerical model of the Gulf of Mexico, it has been observed that these Loop Current intrusion and eddy-shedding events can play a major role in circulation on the continental shelf (Johnson and Perry, unpublished data). They seem to modulate the parabolic, shelfbreak flow, allowing greater interaction of the deep basin with the shelf itself (Oey 1995).

Wind stress (Fig. 4) data measured at NOAA/NDBC buoy #42007, located in the western part of the Mississippi Bight, were obtained from 1991 to 1996. In 1991, unusually large quantities of blue crab megalopae settled on the collectors in the Mississippi Bight. In 1996, extremely low quantities settled. Major differences in the strength and direction of the east/west (alongshore) component of wind stress occurred in the 2 years. In 1996, the alongshore wind stress during spring and summer was anomalously weak, or reversed, as compared to 1991. Using a lag time of 5 to 6 days, Rabalais *et al.* (1995) found a correlation between megalopal settlement at two sites in the Mississippi Bight, one in Mobile Bay and the other in central Mississippi Sound, and suggested a westward alongshore movement of water masses carrying the megalopae as one possible explanation for this relationship. Both numerical models and observations (Johnson and Perry, unpublished data) have demonstrated a characteristic alongshore drift toward the west. Stronger westward wind stress during the spring of 1991 would suggest a transport route for blue crab megalopal reinvasion of estuaries from enhanced alongshore flow toward the west. During the spring of 1996 and during September, commonly the maximum settlement month, the wind stress that could contribute to this flow was absent. This study represents the first attempt to develop a conceptual hypothesis relating physical forcing to dispersion and retention of larvae in the Mississippi Bight.

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NOVEL OPTICAL REMOTE SENSING AND GROUND-TRUTHING OF BENTHIC HABITAT USING THE BURROW-CUTTER-DIAZ PLOWING SEDIMENT PROFILE CAMERA SYSTEM (BCD SLED)

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ABSTRACT The Jefferson Benthic Sled provides video sediment profile imagery of continuous cross-sectional data. Subsurface imaging is achieved by attaching a profile camera prism behind an agricultural plow that extends beneath the plane of the sled skids, slicing through the top 10 to 20 cm of sediment. The plowing video profile provides a high-resolution, real-time, remotely controlled view of the flat side of the plow furrow. Successful continuous profiles of up to 100 m have been collected. The equipment allows immediate characterization of benthic habitats, transition zones, sediment types, sediment oxidation layering, biological resources, and fisheries impact.

KEY WORDS: Sediment profile, underwater video, benthic habitat

INTRODUCTION

Sediment profile imagery has been improved from discrete point data to continuous cross-sectional data by the development of the Burrow-Cutter-Diaz plowing profile camera sled system (BCD sled). The camera plows through the sediments and provides a continuous sediment profile image. Uninterrupted sub-bottom video imaging is achieved by attaching a modified sediment profiling camera prism behind a plow blade that extends beneath the plane of the sled runners and slices through the top 30 cm of sediment. The BCD sled provides high-resolution, real-time imaging of the flat side of the plow furrow as it develops. Successful continuous profiles have been collected that span up to 100 m. Attachment of other equipment allows simultaneous delineation of benthic habitat and near-bottom water conditions. Using the plow-sled, we can immediately characterize benthic habitats, transition zones, sediment types, apparent sediment oxidation layer, and biological resources, assess bottom fisheries impact, and achieve concurrent ground-truthing of side-scan sonar survey data by deploying the plow-sled during sonar surveys.

SYSTEM

The BCD sled (Fig. 1) is configured to collect real-time video from the plowed furrow created by a camera system designed for continuous transect sediment profile imagery. Figure 1 depicts the main components of the plow-sled system: the plow blade and plowing profile camera prism casing with the prism window on

one side, and the two sled runners. The runners ride along the sediment surface, and the lower half of the plowing camera casing travels through the surficial sediments. The plowing profiling camera prism is rotated 90° from the still image sediment profile camera system (Rhoads and Cande 1971, Rhoads and Germano 1982, Germano 1983, Rhoads et al. 1996). The plowing profile camera prism window is flush with the flat, vertical side of an underwater plowing device, and enables viewing the sediments in profile as the plow slices through the uppermost layers (30 cm) of sediment.

The video system and lighting are powered by 12 V battery pack system in an underwater housing. Remote viewing is achieved, presently, through a 150-m long underwater cable. Sled tow transects are continuously logged on the vessel as Global Positioning System (GPS) coordinates. A hydrographic sensor system may be attached to the sled to provide simultaneous continuous acquisition of water depth, temperature, salinity, dissolved oxygen, and pH.

DATA OUTPUT

Composite images, such as the example in Figure 2 (top), from the plowing camera are compiled from digitized versions of Hi-8 video sequences recorded from the vessel during deployment. Additional video and still cameras may be affixed to the sled and to provide close-up plan-view or oblique-view images of the sediment surface. The combination of images, continuous sediment profile imagery, and surface-view images, produces a high-resolution optical analog to acoustic side-scan sonar and sub-bottom profiler images. Postprocessing of digitized video images allows quantification of microtopography, fauna, and sediment attributes in terms of transect data and analyses similar to those described by Malatesta et al. (1992).

The top image in Figure 2 is a mosaic of several video frames acquired from a short section of one sled transect. This reconstruction of the bottom is accomplished by manual registration of adjacent digitized video images using visual surface and subsurface features. Several image features contain information about the substrate properties and the benthic biological community. For example, the sediment-water interface, or seafloor geometry, is visible as the green to yellowish-brown color transition. The yellowish-brown sediment layer represents the apparent color redox-

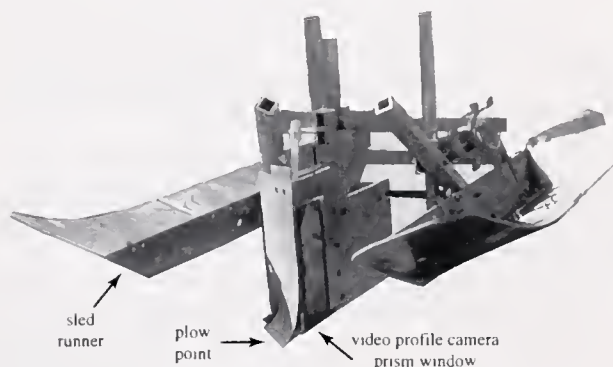


Figure 1. Burrow-Cutter-Diaz plowing profile camera sled.

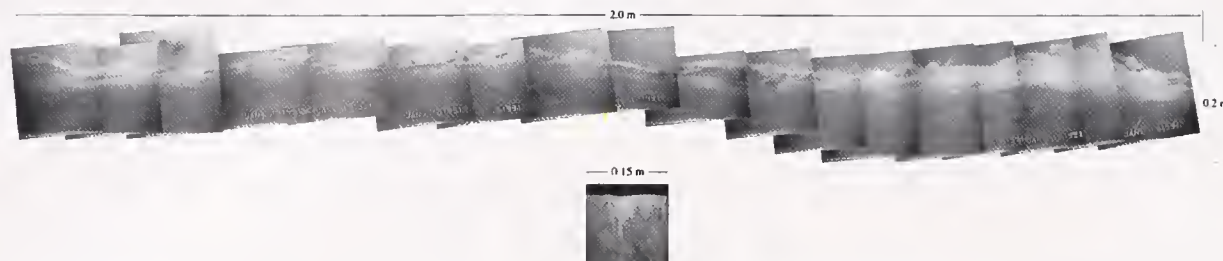


Figure 2. Plowing profile camera image mosaic (2-m wide) above, and standard profile camera image (0.15-m wide) below.

potential discontinuity (Rhoads and Germano 1982, Diaz and Schaffner 1988).

ANALYSIS

Geostatistical methods (Journel and Huijbregts 1978) are applied for analysis of the composite plow image features (Fig. 2, top). Sedimentary and biological habitat features are measured according to Diaz and Schaffner (1988) and Rhoads and Germano (1982, 1986), but at uniformly spaced distance intervals, or lags. Superposition of a grid over the images, such as in Figure 2 (top) and measuring parameters at each intersection with a vertical grid-line produces a series of measurements of the same feature over a range of distance scales. This allows the data to be treated as regionalized variables for the study area rather than a single-valued datum for a sample point, such as is obtained from standard sediment profile image (Fig. 2, bottom). This approach provides data that can be used to quantify the spatial behavior of the parameter, in addition to allowing calculation of over-all mean parameter values for any desired section of the bottom. Habitat features and habitat delineations are accomplished using the geostatistical data obtained from the plow images, and assuming correlations between the sediment profile imagery parameters and habitat properties according to the relationships demonstrated by Bonsdorff et al. (1996).

SYSTEM APPLICATIONS

The primary applications of the BCD sled include benthic habitat delineation and mapping, assessment of benthic living re-

sources, disturbance impact assessment, ground-truthing for side-scan sonar surveys, water quality model parameter evaluation, and thin-layer disposal monitoring.

DEVELOPMENTS

Installing a level or inclinometer in the prism will relate fore-aft inclination angle, allowing bottom image transect reconstruction using sled-tilt angles observed during deployment. The compilation image is analogous to a very detailed acoustic sub-bottom profile images.

Additional video and still cameras may be affixed to the sled to provide close-up plane-view or oblique-view images of the sediment surface. The combination of images, continuous sediment profile imagery and surface-view images, produces a high-resolution optical analog to acoustic side-scan sonar and sub-bottom profiler images. Resolution is now limited by the video signal and video-digitizer. The real-time and assembled plowing video profile images should be very useful to side-scan sonar ground-truthing efforts, enabling rapid determination of sources of variation in the broad-scale acoustic reflection patterns.

ACKNOWLEDGMENT

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MONITORING COASTAL ENVIRONMENTS USING SATELLITE TIME SERIES

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ABSTRACT Databases developed from satellite products can be used to understand long- and short-term oceanographic processes. An automated processing system developed for the creation of co-registered and satellite-derived image databases of sea surface temperature and bio-optical parameters from the Advanced Very High-Resolution Radiometer (AVHRR) and Coastal Zone Color Scanner (CZCS) sensors is presented. We have applied database techniques toward predicting fish locations for the West Coast of the United States. We present a user interactive graphical interface to display the databases visually and to connect a series of fish behavior rules by performing logical operations. This program provides a real-time interactive display for testing and developing an understanding of fish behavior and location.

KEY WORDS: Time series, satellite, sea surface

INTRODUCTION

The coastal environment represents a changing and complex environment where a variety of forcing mechanisms influence the physical and bio-optical processes. Local winds and tidal mixing processes occur on short time scales, producing changes in coastal properties (temperature, salinity, optics). Unfortunately, traditional shipboard sampling programs are inadequate to characterize these properties. Satellite remote sensing provides a unique capability to address the spatial variability of these properties when they are integrated with shipboard measurements programs. Improved satellite products are under development to provide daily updates of the coastal environment. Previous studies have used remote sensing imagery to address single sporadic events occurring in the coastal region. Our research has extended these efforts to examine a time series of imagery (monthly/short time and yearly/long time) covering the coastal environment to define trends. Because a satellite time series provides a broader temporal context within which to interpret individual events, this approach provides a unique understanding of whether specific events are "typical" or "atypical."

Our objective is to exploit the use of satellite-derived ocean properties using an image time series analysis to monitor coastal processes. We developed a software package, Automated Processing System (APS), to aid in establishing a time series of imagery. The time series will consist of sea surface temperature (SST) and surface bio-optical products using historical, present, and future thermal and ocean color sensors. Presently, our time series contains historical bio-optical products (chlorophyll, k490, etc.) developed from the Coastal Zone Color Scanner (CZCS) at 1- and 18-km ground pixel resolution and historical and present SST developed from the Advanced Very High-Resolution Radiometer (AVHRR) at 1-km ground pixel resolution. In the near future, we will be adding to our time series using new ocean color sensors (Sea-Viewing Wide Field-of-view Sensor (SeaWiFS) HRST, Moderate Resolution Imaging Spectroradiometer (MODIS), etc.) as the data become available. We applied statistical analyses on these time series products to: (1) investigate climatological trends and temporal and spatial variability in coastal regions; (2) develop, advance, and validate old and new algorithms by integrating *in-situ* measurements; (3) develop behavior rules to link fish distributions with the time series products; and, finally; (4) develop a Graphical User Interface (GUI) to aid in the prediction of fish locations based on the behavior rules.

THE AUTOMATED PROCESSING SYSTEM (APS)

The Automated Processing System (APS) was developed for the unattended creation of co-registered, satellite-derived image time series. The APS provides a method to reprocess large image datasets with new algorithms and updating databases with new products (Martinolich 1996). The APS contains programs for sensor calibration, atmospheric correction, and geometric registration in addition to using standard algorithms for the generation of level-3 products geographically modified into a Mercator projection. The level-3 products are accessible to the World Wide Web via the NRL Code 7240 home page (<http://www.7240.nrlssc.navy.mil/ocolor/>). The APS was developed using C and FORTRAN on a silicon graphics workstation and will be upgraded to handle future sensors. The APS receives real-time imagery using a Terascan receiving system (High Resolution Picture Transmission (HRPT)) and converts the pass to the standard level-1b format, raw telemetry with appended earth location and calibration. Next, the level-1b files are transferred via ftp to a silicon graphics challenge ("ln" directory) where the APS monitors the input directory for new level-1b data. Once the APS recognizes that an unprocessed file is there, it moves the file to the "work" directory, where the images are processed to level-3 products. Level-3 products are generated for multiple regions (developed by the user) during the processing. Each time the APS processes a level-1b scene, it looks in the "areas" directory and checks whether the scene covers an area of interest. If so, a level-3 product is produced for that area; if not, the next area of interest is checked until all areas have been checked and processed. The level-3 products are automatically transferred to the time series database, and a Graphics Image Format (GIF) file is created for quick viewing. After processing is complete, the level-1b file is moved to the "out" directory to be archived. Currently, the time series database occupies 12 gigabytes of on-line disk storage. Figure 1 is a flowchart showing the structure, data flow, and functionality of the APS.

Presently, we use the APS to create a time series for AVHRR imagery. The AVHRR level 3-products consist of sea surface temperature (SST) and c 660 (turbidity). The turbidity product is used primarily in coastal waters because of a weak signal in offshore waters. It is derived from an empirical algorithm using AVHRR channels 1 and 2, after atmospheric correction (Gould and Arnone

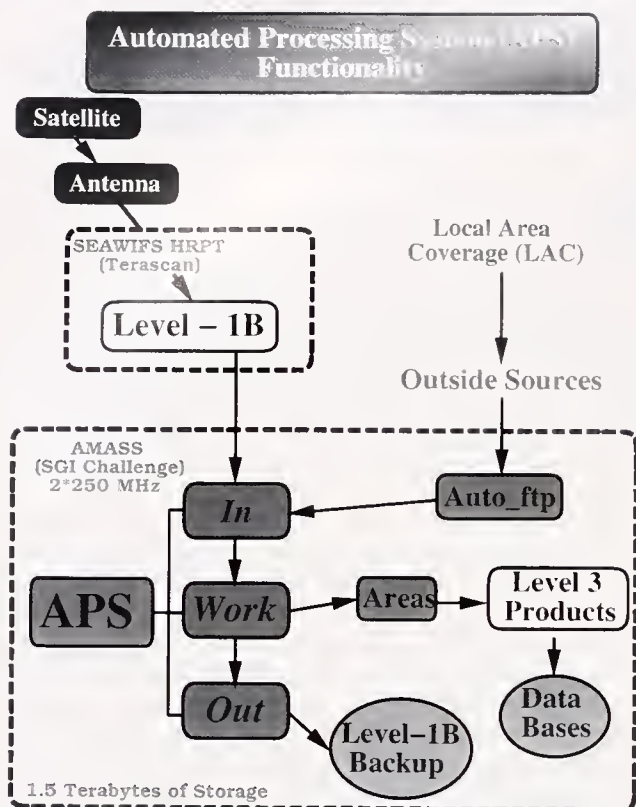


Figure 1. Flowchart showing the basic structure, data flow, and functionality of Naval Research Laboratory's Automated Processing System (APS).

1997). The c 660 algorithm yields excellent results in turbid coastal waters and is extremely valuable in the summer months when AVHRR SST values are homogeneous. The AVHRR areas of interest for real-time processing cover the Gulf of Mexico (April 1994 to present), Mississippi Bight (January 1997 to present), Mississippi Sound (January 1997 to present), San Diego/Gulf of California–West Coast U.S. (August 1995 to present), Chesapeake and Onslow Bay–East Coast U.S. (August 1994 to present), Arabian Sea (February 1994 to December 1995), Sea of Japan and Yellow Sea (June 1996 to present). The geometric registration used for the AVHRR time series is Mercator. Figure 2 illustrates all regions currently processed for AVHRR, CZCS, and planned SeaWiFS. Figure 3a is an example of the AVHRR SST for Gulf of Mexico on March 19, 1995. Notice that the Loop Current in the SST image is being divided and pinched off in mid-Gulf of Mexico. The lighter colors represent cooler surface waters and the darker colors warmer waters. Figure 3b is a comparison of the SST and c 660 for the Mississippi river outflow region for April 7, 1997. Notice the Mississippi river turbidity plume. The red, orange, and yellow colors represent highly turbid waters; whereas, the green, blue, and purple colors represent clearer, nonturbid waters. Notice how much more the coastal features are seen in the turbidity image during a time when the SST is homogeneous.

The APS was also used for creating Mercator-registered image time series for the historical Coastal Zone Color Scanner (CZCS) sensor (1978 to 1986) at 1 km resolution. The CZCS level-3 products consist of diffuse attenuation coefficient at 490 nanometers (K_{490}) and chlorophyll (Feldman et al. 1989). The atmospheric correction was performed using the Gordon (Gordon et al. 1983) algorithm, and a coastal iteration of the water-leaving radiance at 670 nm was applied using a technique developed by Gould and Arnone (Gould and Arnone 1994). The iteration is required in

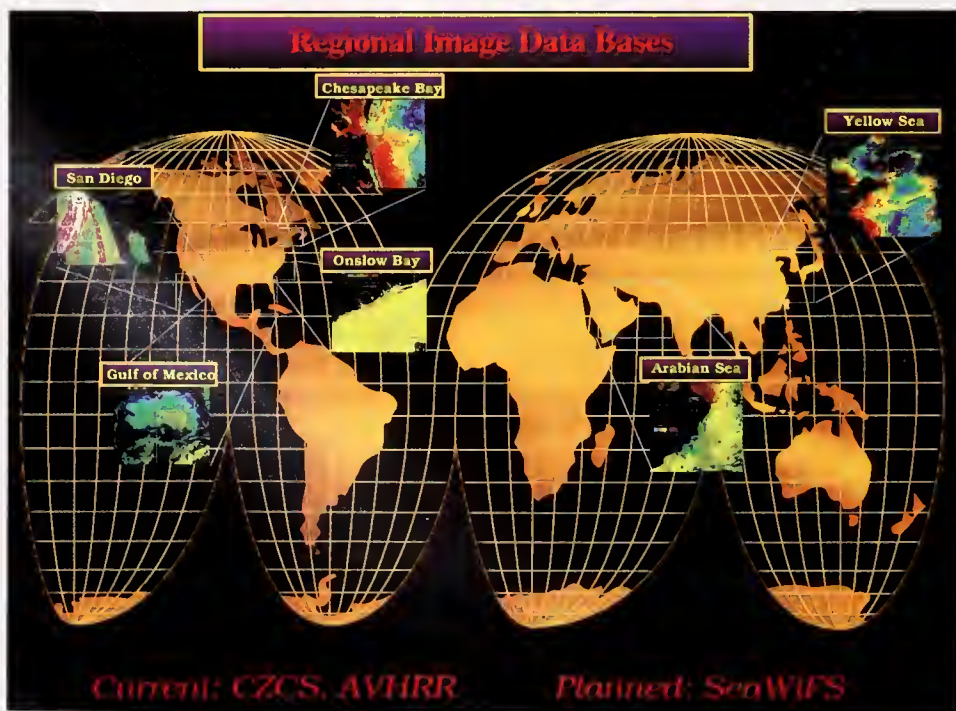


Figure 2. Illustration of all regions that are being processed by NRL Code 7240 and time series regions for AVHRR, CZCS, and planned SeaWiFS.

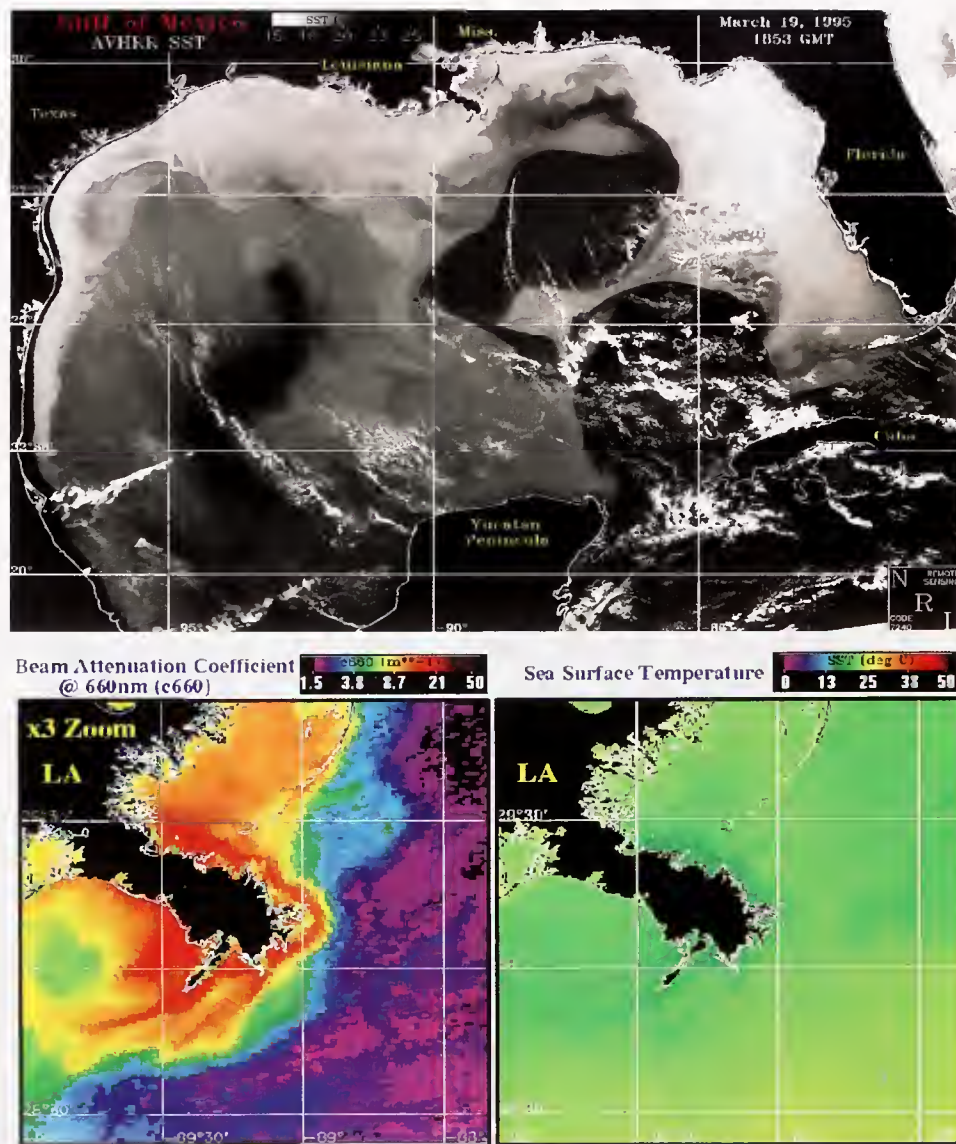


Figure 3. (a) AVHRR SST example of the Gulf of Mexico region from March 19, 1995; (b) AVHRR SST versus c 660 "turbidity" comparison for April 7, 1997 for the Mississippi river outflow region.

coastal waters to improve the atmospheric correction in coastal regions. The time series consists of the diffuse attenuation coefficient, K_{490} , product (Austin and Petzold 1980), and the phytoplankton pigment concentration (Gordon et al. 1983). The CZCS time series include the Gulf of Mexico (November 1978 to June 1986), Arabian Sea (November 1978 to June 1986), and Sea of Japan and Yellow Sea (November 1978 to June 1986). Figure 4a,b illustrates examples of the K_{490} in the Arabian Sea/Gulf of Oman region for April 4, 1979 and phytoplankton pigments in the Sea of Japan region for mean of June 4 to 5, 1980. The red, yellow, and orange colors represent high K_{490} and pigment values; whereas, the green, blue, and purple colors represent lower values. The importance of the long time series is that it provides trends in the coastal properties that are not always observed in individual images. Individual images from AVHRR and CZCS are limited by cloud contamination, which many times limits our ability to see trends clearly. By analyzing numerous images, we can effectively observe trends in ocean properties.

TIME SERIES STATISTICAL ANALYSES

Regional means and standard deviations derived from statistical time series analyses are used to assess trends in coastal and shelf optics. We developed an analysis program written in PV-WAVE to examine spatial and temporal scales of variability and seasonal/annual trends from monthly and annual image climatologies. Time series supply valuable baseline information for certain geographical locations/regions of interest, including a synopsis of temporal and spatial variability leading up to field sampling and information for algorithm development and testing. Figure 5 is an example time series analysis showing the mean and standard deviation of turbidity (c 660 parameter) for three regions of interest in Mobile Bay, Alabama (La Viollette et al. in press). From April 1995 to March 1996, the program averages each pixel within defined blotch areas for each image and outputs file name, number of valid pixels under blotch, mean, standard deviation, and the coefficient of determination (La Viollette et al. in press). Notice the

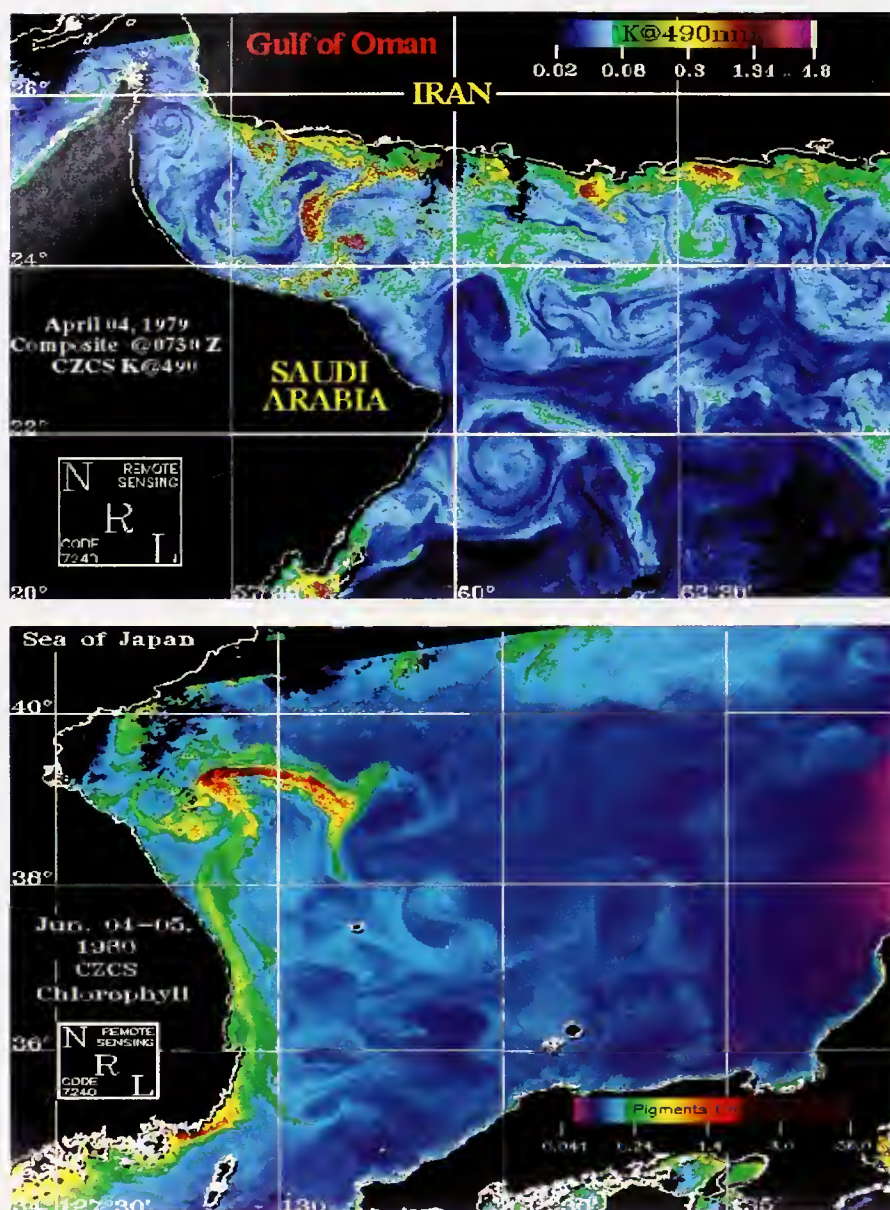


Figure 4. (a) CZCS K490 example of the Arabian Sea/Gulf of Oman region from April 4, 1979; (b) mean CZCS chlorophyll example of the Sea of Japan region from June 4 to 5, 1980.

elevated turbidity in Mobile Bay from June to March 1996, which reflects the heavy rains resulting in an increase of river runoff during the spring months.

FISH PREDICTION

We have developed a software tool to utilize a variety of databases to predict distribution patterns of individual fish species off the U.S. West Coast. Fish biomass observations were combined with climatology of satellite derived ocean products at 18 km resolution to interactively determine relationships and fish behavior interactively. We used aerial fish spotter data obtained from the National Marine Fisheries Service (NMFS) in La Jolla, California. The chlorophyll pigment concentration database was produced from CZCS at 18 km resolution and obtained from Goddard Space Flight Center. The SST database was processed

from AVHRR and obtained from the NASA Ocean Data System (NODS) archives. The 18-km bathymetry was developed from the Synthetic Bathymetric Profiling System (SYNBAPS) using PC-Seapak software (Firestone et al. 1989). The seasonal Mixed Layer Depth (MLD) database was derived from the Generalized Digital Environment Model (GDEM) (Arnone et al. 1995). SST and chlorophyll pigment gradient fields were developed to locate SST and pigment fronts. The fish location data were used in conjunction with the oceanographic climatologies to develop behavior rules for individual species (Ladner et al. 1996) to examine how fish catch is associated with surface ocean properties. An interactive GUI (Fig. 6) was developed to display ranges of ocean products that are associated with certain fish species visually. For example (Fig. 6), tuna prefer SSTs ranging from 25 to 26°C, chlorophyll pigment ranges between 0.16 and 2.5 milligrams per cubic meter, water depth range of 1,700 and 4,100

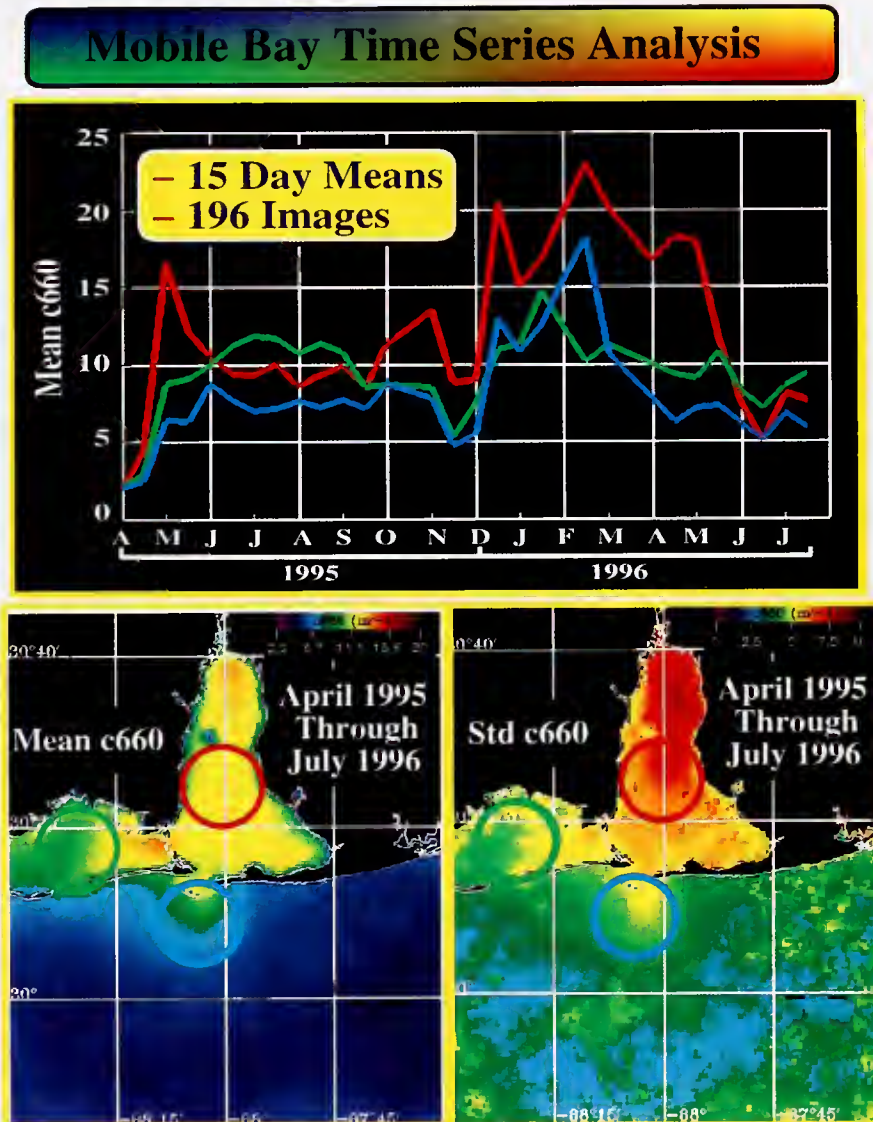


Figure 5. Time series analysis example of the mean and standard deviation to investigate the temporal and spatial variability of c 660 "turbidity" for three regions of interest in Mobile Bay, Alabama from April 1995 through March 1996.

feet, MLD between 0 and 50 m, and a chlorophyll pigment gradient between 0.0 and 0.05 milligrams per cubic meter. Fish locations are based on the behavior rules developed from the spotter data and the satellite products. Correlations between actual fish distribution and biomass with several ocean parameters demonstrated that biomass for certain fish off the West Coast of the United States was correlated with the ocean parameters. The widget (Fig. 6) allows the user to set the ranges of ocean parameters, set desired color, turn windows on/off (small windows) using the sliders and quickly see where all the behavior rules are met. The large window shows the predicted areas of highest abundance for the specified fish species.

SUMMARY

Time series analyses using satellite imagery provide a significant contribution toward characterizing the complex ocean processes occurring in the coastal environments. The enormous

amount of data provided by satellite sensors can be exploited to understand trends of physical and bio-optical processes that might affect shell fish. We have presented methods to organize satellite data into time series that can be used to develop climatologies and statistical analyses of these data. This research has automated procedures to characterize the SST and optical variability for a number of coastal and open ocean regions around the world. We have applied these techniques to several sensors and have constructed database products.

Time series analysis aids our understanding of the spatial and temporal variability of the coastal region. Statistical relationships from the time series have illustrated seasonal and interannual trends in the SST and bio-optical processes. Our plans are to integrate shipboard data with these time series to define the coupling processes better.

The time series analysis of satellite products can be exploited to further understanding the regional distribution of fish biomass. We presented an example of the use of satellite-derived products to



Figure 6. Predicted January 1983 tuna distribution (large window) based on an intersection of fish behavior rules with the ocean properties seen in the small windows. The fish prediction interactive widget visually displays the ocean product and prediction results. It also allows the user to set the ranges/behavior rules using the slider bars under each window. Also, the user can select a desired color for each window, turn window on/off, etc.

delineate trends in fish distribution off the U.S. West Coast. Although these results require additional validation, the methods to characterize and monitor the coastal environment have been clearly demonstrated.

The methods and techniques we describe for rapid analysis satellite imagery using time series are being applied to new satellite systems. We are at the verge of a new era in satellite imaging

of Earth's surface. A series of new satellites is planned for the next few years (MODIS, HRST, EPA3, MOS, etc.), and SeaWiFS, which was launched in June 1997, is being implemented in a time series analysis. Our previous research suggests that new products and algorithms will emerge from these new sensors. Future research will integrate time series analysis of satellite products into routine monitoring of the coastal environment.

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GIS AND MODELING: COUPLING HABITATS TO FLORIDA FISHERIES

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ABSTRACT To fill the need for information relating fishery species to habitat and anthropogenic effects on marine ecosystems, the Florida Marine Research Institute created the Marine Resources Geographic Information System (MRGIS). This comprehensive, coastwide database for research and management of fisheries provides spatial information for siting of clam aquaculture, hard-bottom mapping, and fishery exclusion zones. Fisheries-independent monitoring for Florida estuaries and habitat suitability modeling are also being used to create predicted distribution maps of fish and invertebrate species. Four case histories are summarized, designed to define relationships between habitat and marine fish and invertebrate species in Florida.

KEY WORDS: Fisheries habitat, habitat suitability modeling, essential fish habitat, geographic information systems, fisheries management

INTRODUCTION

The Florida Marine Research Institute (FMRI), a bureau within the Florida Department of Environmental Protection (FDEP), is largely responsible for providing the estuarine and coastal marine information used by fisheries and environmental managers. FMRI has adopted a holistic approach for the monitoring and research needed to manage coastal marine ecosystems. The Coastal and Marine Resources Assessment (CAMRA) group at FMRI has created the Marine Resources Geographic Information System (MRGIS) to support studies in which geographic information systems (GIS), remote sensing, and modeling are used to link the seascape to marine resources. This paper summarizes four case histories using spatial methods designed to explore the relationships between habitat and marine fisheries in Florida.

METHODS AND RESULTS

Hard-Clam Aquaculture

The Florida legislature recently enacted legislation to promote aquaculture development (FDACS 1996). Clam aquaculture has become popular in Florida because of the high growth rates and consequent rapid crop turnover. Hard clam (*Mercenaria mercenaria* and *M. campechiensis*) farming is Florida's most rapidly emerging form of aquaculture, with \$5.4 million in sales by 142 growers in 1995. This compares with \$1.2 million in sales by 41 growers in 1991. The Florida Department of Labor and Employment Security recently funded expanded programs to teach clam and oyster culture techniques to former commercial fishermen. Hard-clam aquaculture is expected to continue expanding, provided applicants can obtain suitable lease sites on state-owned submerged lands. FDEP is responsible for overseeing the assignment and monitoring of submerged lands leased for hard-clam aquaculture.

A recently developed fishery for hard clams exists in the Indian River and Mosquito Lagoon in eastern Florida. FMRI scientists have developed methods using GIS to identify submerged lands

suitable for hard-clam aquaculture (Arnold et al. in press). The GIS coverages contained in the MRGIS, pertaining to habitat type (e.g., seagrass, bathymetry), distribution of biological resources, proximity to transportation corridors, and other human-use facilities were used, along with site-specific information concerning clam abundance from field surveys. GIS is being used to ascertain lease-site suitability, size, and layout in the Indian River/Mosquito Lagoon complex (Fig. 1). This approach reduces the uncertainty associated with selecting lease sites by addressing potential conflicts between user groups and helps to plan the spatial extent and growth of the Florida hard-clam aquaculture industry. The resultant maps can assist managers to determine general areas suitable for hard-clam leasing and help those applying for permits to choose potential lease sites.

Hard-Bottom Mapping

Marine fisheries scientists, user groups, and resource managers in the southeastern Atlantic states have identified that there was a need for accurate information about the location and extent of hard-bottom habitat generally associated with reef fish stocks. To address this, a Bottom Mapping Working Group was formed in 1985 by the South East Area Monitoring and Assessment Program management committee (Perkins et al. 1997). The work group met to establish a bottom-mapping database using information from a variety of surveys. The initial study was summarized in reports by scientists from South Carolina, Georgia, and North Carolina (Van Dolah et al. 1994, Moser et al. 1995). A total of 23,960 records of information on location and bottom type were compiled.

The Florida study group was initiated in 1994 (Perkins et al. 1997). It has: (1) expanded the list of hard-bottom obligate fishes to 264 taxa; (2) developed a protocol for using information gathered from specimen collections to acquire evidence of bottom type; and (3) developed a protocol for incorporating geographic data, so that the area data records are equivalent to the point and line-segment records added during the first two parts of the study. Florida added 20,787 records to the database from 37 sources that had bottom-type determinations. Of these records, 900 were de-

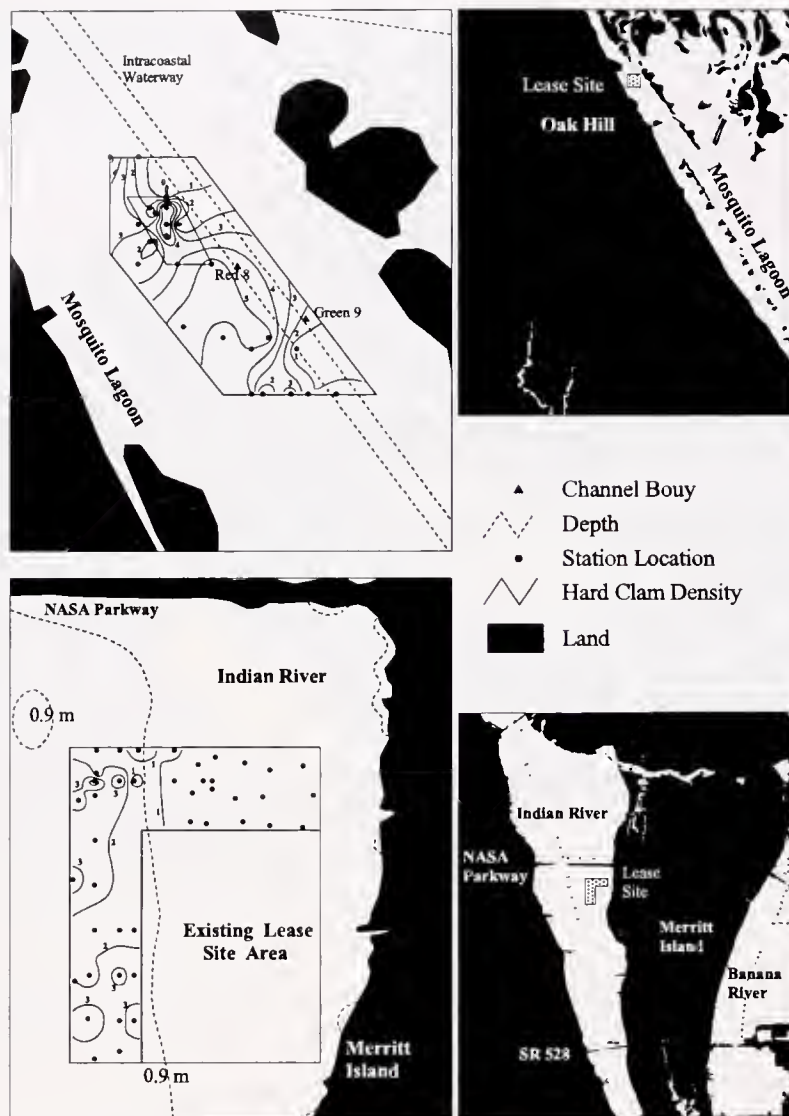


Figure 1. Indian River and Mosquito Lagoon area maps showing the current hard clam lease sites; W. White, FMRI.

rived from nine databases developed using side-scan sonar. The GIS database created contains fish and invertebrate data and physical bottom-type information obtained from aerial photography, benthic grabs, dredges, trawls, traps, vibracore samplers, scuba observations, submersibles, recording fathometers, side-scan sonar, and subbottom profilers.

The FMRI scientists identified polygon GIS data as another type of information that could be included in the bottom-mapping database (Perkins et al. 1997). A grid composed of 1-minute-square cells was created that extended from the Georgia border to south of Jupiter Inlet off east-central Florida. ArcView 3.0 and ArcPlot in the Arc/Info GIS were used to display a rasterized version of the point, line, and polygon data. Bottom type was assigned to each grid cell for which there were data. Most areas represented by polygons were surveyed with combinations of gear (fathometer and video, bottom profiler and fathometer). Maps summarizing bottom-type determinations were then created. Determination of bottom type was recorded in the Florida database for approximately 37% of the grid cells. Of these, 713 were coded

as hard bottom, 405 were coded as probable hard bottom, and 3,042 were coded as "no evidence of hard bottom" (Fig. 2). The data indicated that the area with the greatest percentage of hard bottom, plus probable hard bottom near shore, is in the southern part of the study area. The second highest percentage is in the northern part of the study area.

The GIS database also allows the identification of quadrates in which the coral *Oculina varicosa* and Coquinoid-Rock/Sabellariid Reefs have been recorded (Perkins et al. 1997). The *Oculina* reefs provide a very important habitat for hard-bottom fishes, and fishing is currently prohibited in a large part of this area. The dominant polychaete worm that helps build the sabellariid reefs is *Phragmatopoma caudata*. It builds tubes in large, subcylindrical colonies about 1-m in diameter on hard substrate in high-energy beach areas of southeastern Florida. Colonies of the worm are known to occur from south of Key Biscayne north to Cape Canaveral. The geographic extent of the sabellariid reef habitat determined was much greater than originally anticipated. The database created in ArcView can be queried to determine the surveys, the types of bio-

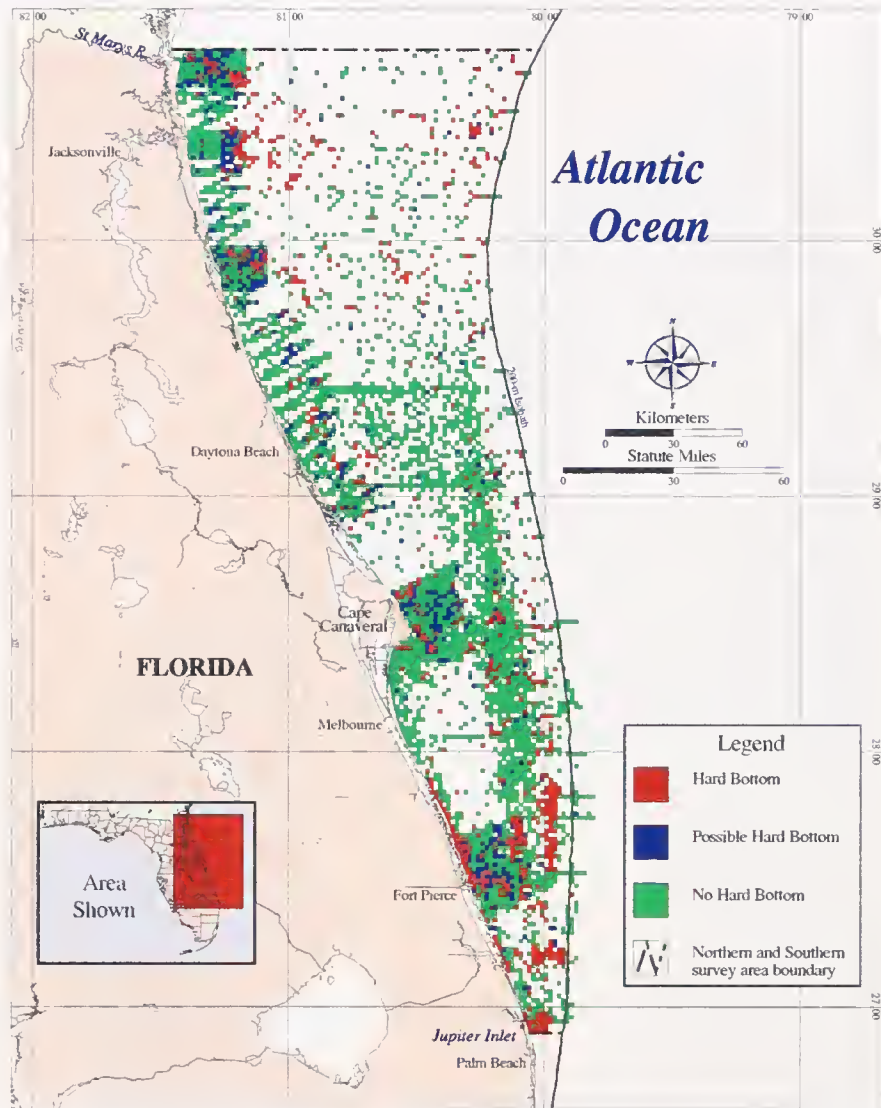


Figure 2. Distribution of grid cells in the Florida survey area that contain information on bottom type; D. Wilder and H. Norris, FMRI.

logical information used to infer the bottom type, and/or the physical-oceanographic studies used to determine bottom topography.

Shrimp Management Plan

The Florida Marine Fisheries Commission (FMFC) has used Resource Impact Maps (RIM) produced by CAMRA to assist with the implementation of a shrimp management plan for five regions statewide (Haddad et al. 1993). The first phase of the plan addressed allowable gear type, mesh size of nets, and the shrimp count for harvesting activity. Numerous local laws were repealed, which simplified inshore and nearshore shrimp regulations and standardized the shrimp fishery on regional and statewide levels. The second phase of rule-making addressed the issues of finfish bycatch associated with shrimp trawling and the adoption of a zonal management plan to determine areas in which shrimp harvesting areas would be allowed.

The Big Bend Region was one of the first areas where the FMFC examined the need to establish harvesting areas for pink

shrimp (*Penaeus duorarum*) in state waters. Extensive consultations with live-bait fishermen, food-shrimp fishermen, and recreational fishermen were conducted to determine the areas used in their fisheries. The RIM maps were used to compare areas utilized by the pink shrimp fisheries with the geographic distributions of benthic habitats in the MRGIS (Poehlman and Westlake 1995, Steele and Norris 1995). Maps showing proposed closure zones for pink shrimp (Fig. 3) were prepared and periodically updated by CAMRA during the consultation process. The maps produced using GIS allowed the FMFC to explain complex issues to all concerned parties. Comparisons assisted fisheries managers in making decisions that would protect critical nursery habitats while reducing conflicts between various user factions within the commercial and recreational fisheries.

Seagrass beds represent one of the most productive and important habitats in the nearshore marine systems of Florida. They are used by a wide range of species as feeding grounds, nurseries, and refuges from predation. In the Big Bend Region, shallow-water

Institution from 1991 to 1994. It currently summarizes data on the seasonal abundance of 91 fish and invertebrate species by life stage, environmental occurrence, and habitat associations. It is based on qualitative and quantitative information obtained from scattered published and unpublished literature. It consists of textual and numeric categorical data tables for Tampa Bay, Sarasota Bay, Florida Bay, and the Indian River estuary.

The textual Species Life History (SLH) table summarizes information on species throughout the northern Gulf of Mexico and the Atlantic coast from Florida to North Carolina, with emphasis on Florida literature (Rubec et al. 1997). There are six types of numeric tables that summarize the seasonal abundance, social and economic importance, and habitat requirements by life stages for each species. The Habitat and Attributes tables are repeated for each of the five life stages.

The FLELMR database is being expanded to include a more comprehensive SLH outline. Textual information will be retrieved by topic. The habitat associations of species life stages will be summarized in Benthic Habitat and Water-Column Habitat tables. Suitability Index tables will summarize monthly abundance rankings across environmental gradients. There are nine textual table types and 18 numeric table types associated with single species for five geographic regions. It also will feature 12 textual benthic habitat tables associated with 12 fish species diversity tables for habitat zones within each region. Most of the textual and numeric tables have the same headings to facilitate relating information. FLELMR is designed so that a compiler can summarize information from the literature into textual tables. Key environmental parameters are then transferred to the numeric tables. All tables have windows for citation numbers that tie to the master bibliography. FLELMR is evolving toward a spatial decision support system (SDSS) in which maps of species distributions by life stage are linked to the textual and numeric tables in the new database. Species distribution maps will be created using Habitat Suitability Index (HSI) modeling.

Habitat Suitability Index Modeling

FMRI and NOAA's Strategic Environmental Assessment (SEA) Division are collaborating to evaluate HSI modeling as a technique to determine species distributions in areas where no fisheries-independent monitoring is conducted. FMRI's goal is to map the geographic distributions of young-of-year, juvenile, and adult life stages of approximately 50 species of marine fish and invertebrates in 18 Florida estuaries over the next few years. The fish and invertebrate species distribution maps will be used for oil spill response and fisheries management.

Scientists from FMRI and SEA Division are working together to model and map the geographic distributions of fishery species by life stage and season (Rubec and McMichael 1996, Rubec et al. 1997). Various techniques have been applied to relate abundance of fish or invertebrates to environmental gradients for use in HSI models (Monaco and Christensen 1997, Monaco et al. 1998). SEA Division recently used GIS to analyze and map predicted biological distributions in Pensacola Bay, Florida using data from FMRI and other sources (Christensen et al. 1997a, Christensen et al. 1997b). Suitability indices (SIs) across environmental gradients for juvenile and adult spotted seatrout (*Cynoscion nebulosus*), eastern oyster (*Crassostrea virginica*), and white shrimp (*Penaeus se-*

tiferus) from Pensacola Bay were derived from the scientific literature.

FMRI and SEA Division scientists are currently analyzing fisheries-independent monitoring data from Tampa Bay, Charlotte Harbor, Choctawhatchee Bay, Florida Bay, and the Indian River estuary to relate catch-per-unit-effort (CPUE) of fisheries and forage species to habitat-related factors. Suitability Index curves are being determined to define which portions of environmental gradients are most important in explaining species abundance. Biological and hydrological data also are being subjected to multivariate analyses to classify the community of fishes associated with various portions of environmental gradients (Bulger et al. 1993). Biologically relevant ranges of salinity, temperature, depth, dissolved oxygen, sediment type, and benthic vegetation type can then be interpolated and stored in the MRGIS. The resulting habitat maps will summarize seasonal changes in salinity zones (caused by fluctuations in freshwater inflow) and water temperature zones.

HSI Modeling Procedure. HSI models access SIs associated with the interpolated habitat layers stored in the database. Avenue language macros run the HSI model with the ArcView 3.0 GIS using the Spatial Analyst module developed by Environmental Systems Research Institute. The data layers representing different habitats are overlaid in the database. The model uses rasterized grid layers with a cell size of 100 by 100 meters (Fig. 4). The HSI formula calculates a composite SI coefficient by taking the geometric mean of the SI values representing the habitat layers for each grid cell. After all the cells across the estuary are evaluated, a HSI map is produced that depicts the geographic distribution and relative abundance zones for each species life stage.

Verification. Current HSI work is focused on predicting the spatial distributions of bay anchovy (*Anchoa mitchilli*), spotted seatrout, (*Cynoscion nebulosus*), and pinfish (*Lagodon rhomboides*), by life stage and season in Tampa Bay and Charlotte Harbor (Rubec et al. 1998).

HSI modeling was used to create predicted maps of the most suitable zones for juvenile spotted seatrout in Charlotte Harbor (Rubec et al. In press). Mean SIs within zones in Charlotte Harbor were compared with mean CPUE data to demonstrate increasing abundance of juvenile spotted seatrout in more suitable habitats. Ranking and statistical testing demonstrated a higher affinity of juvenile spotted seatrout for shallow areas with submerged aquatic vegetation.

Transferability of Suitability Indices. A second series of HSI models are being tested using habitat layers from each estuary, and SIs reciprocally transferred between Charlotte Harbor and Tampa Bay (Rubec et al. 1998). The verification procedures are repeated to compare mean SIs with mean CPUEs within predicted zones. Using these techniques, we hope to determine the statistical reliability of HSI modeling and mapping involving transferred SI values. The goal is to transfer SIs to predict species distributions in estuaries, where no fisheries-independent monitoring is conducted.

DISCUSSION

Fisheries biologists associated with fisheries management councils nationwide are currently trying to amend fisheries management plans so that they include the identification of essential

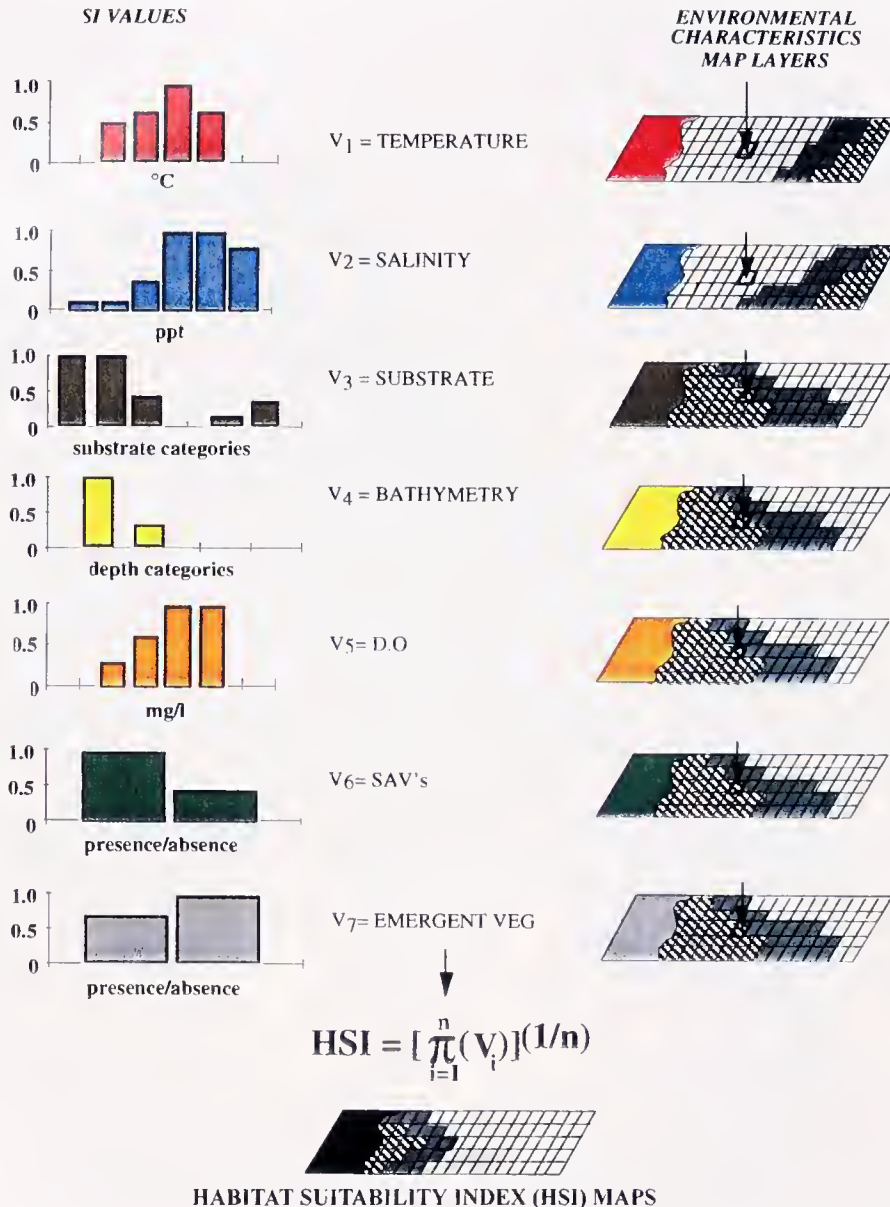


Figure 4. Grid-based habitat suitability index modeling to predict fish distributions by life stage and season; J. Christensen, NOAA SEA Division.

fish habitat (EFH) mandated in the Magnuson–Stevens Fishery Conservation and Management Act (Rubec et al. 1998). EFH was defined as “those waters and substrate necessary for spawning, breeding, feeding, or growth to maturity.” The councils recognize the need to use GIS to define estuarine and marine habitats spatially. However, it is unclear how the councils will decide which habitats are “essential.”

The councils must develop objective methods to identify EFH. The FLELMR SDSS under development will help to identify the environmental requirements and habitat associations of estuarine species in Florida (Rubec et al. 1997). HSI modeling can be used to predict zones of low to optimal habitat suitability across an estuary. HSI models also can be used to run scenarios that can assess the potential effects of anthropogenic changes. The zone of optimal abundance predicted from the model could be interpreted

to be an EFH Habitat Area of Particular Concern (Rubec et al. 1998). Hence, summaries of scientific literature and HSI maps in the FLELMR SDSS can provide the scientific framework needed to define and delineate EFH in Florida estuaries (Rubec et al. 1997).

Haddad (1997) noted that management of marine fisheries habitat has historically been a secondary concern of state and federal fisheries management councils and commissions. Fisheries managers are beginning to focus better on fisheries–habitat issues. The mandate to conserve habitat is becoming clearer as the concepts of ecosystem management are put into practice. Rubec (1996) noted that the traditional approach involving narrowly focused legislation (top-down control) being practiced by different management agencies is inadequate. Stewardship involves a bottom-up approach, wherein stakeholders and managers discuss and

agree upon the problems in the context of the entire ecosystem. Sampling, data management, and GIS-based modeling should be

coordinated between agencies to promote informed ecosystem management.

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APPLICATION OF AN ACOUSTIC SEA FLOOR CLASSIFICATION SYSTEM FOR BENTHIC HABITAT ASSESSMENT

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ABSTRACT Hydroacoustic processors that assess components of acoustic signals reflected from the seabed can provide much information about the character of the seabed. One such system, *RoxAnn*, utilizes acoustic pulses from traditional fathometers/echosounder to generate seabed classification data that can be superimposed onto a navigation system by color coding a vessel's movement according to seabed type. Consistent mapping of an area is achievable without subjective data obtained by grab samples, cores, or diver reports. Data obtained is of a format easily incorporated into a flexible digital environment and links to the navigational system of the vessel.

KEY WORDS: Seafloor classification, remote sensing, hydroacoustics

INTRODUCTION

Knowledge of the seabed is a requirement for many scientific disciplines and commercial activities. Whether the knowledge required is of the geology of the seabed, the biological life forms present, or to assess the impact of human activities, the knowledge must be acquired by technology that is efficient, effective, objective, consistent, and precise. *RoxAnn* is a hydroacoustic processor that achieves all of these demands by remote sensing of the acoustic signals reflected from the seabed. The information generated can be superimposed onto a navigation system by color coding a vessel's movement according to seabed type. Consistent mapping of an area is achievable without subjective data obtained by grab samples, cores, or divers reports.

These traditional methods of lowering tools onto the seabed and removing a portion of the seabed for examination still have their place in seabed classification. Video cameras are currently used in limited numbers of applications primarily because of the expense of deployment and recovery. In addition, the images obtained tend to be subjective. Divers are again used in limited applications not only because of cost restraints but again because of the subjective nature of the results obtained. Processing of acoustic signals transmitted from near the seabed to reflect off the seabed surface and its underlying layers is vastly more sophisticated, but can still be as subjective as the older methods. The images obtained by these techniques require expert interpretation by experienced individuals.

Seabed discrimination requires a technique that fulfils a number of criteria. Ideally, it should be remote, objective, accurate, automatic, unambiguous, efficient, and produce results that are repeatable. Resistance to weather and sea conditions and adaptability are essential qualities.

RoxAnn is field proved and represents a genuinely significant advance in remote sensing technology. This system connects directly across the transducer of a single-beam echo sounder in parallel and is tuned to the frequency of that particular model of echo sounder. This connection does not compromise the echo sounder operation and no additional work through the hull is required. It can be configured to the user's specifications. Data obtained is of a format easily incorporated into a flexible digital environment and links to the navigational system of the vessel. The data generated

by *RoxAnn* are numeric, unambiguous, and objective and can be directly processed into color-coded results against geographical position, depth, and displayed in either two dimensions or as a three-dimensional image.

PRINCIPLES OF ROXANN OPERATION

The entire system incorporates a geographical positioning system, echosounder, computer and software, and the key component, the ultrasonic processor *RoxAnn* (Fig. 1). The relevant acoustics theory involved combines knowledge from three areas of underwater acoustics that are usually discussed separately as seabed (surface) scattering, seabed scattering, and sub-bottom reverberation.

An echosounder operates at frequencies between 15 and 210 kHz. Nominally vertical transmission pulses are emitted from a hull-mounted transducer, and the returns are processed for display in a variety of formats. Assuming that the transducer has its axis (and conical beam pattern) vertical, the first sound waves to return to the transducer after the transmitted pulse are those reflected at normal incidence from the seabed directly under the transducer (Fig. 2).

As the sound travels normally into the seabed, discontinuities at different depths in its structure cause further echo pulses to be reflected, passing through the seabed surface and returning to the transducer as a train of echoes, following that of the initial seabed reflection. This is known as sub-bottom reverberation.

However, a transducer emits sound waves over a wide range of angles, which will impinge obliquely on the seabed. If the seabed were perfectly flat, there would be no returns to the transducer, because the pulses would continue to travel away from the transducer. A significant proportion of the original energy returns directly to the transducer because the seabed is not perfectly flat. This indicates the essential roughness of the seabed (in combination with the hardness measured by the sub-bottom reverberations).

The second echo derives from those beams that have been reflected twice at the seabed and obliquely back-reflected from the sea surface (Fig. 3). The reflection at the seabed gives a direct measurement of the characteristic acoustic impedance of the seabed relative to that of the seawater above it. The characteristic acoustic impedance is a product of the density and the speed of

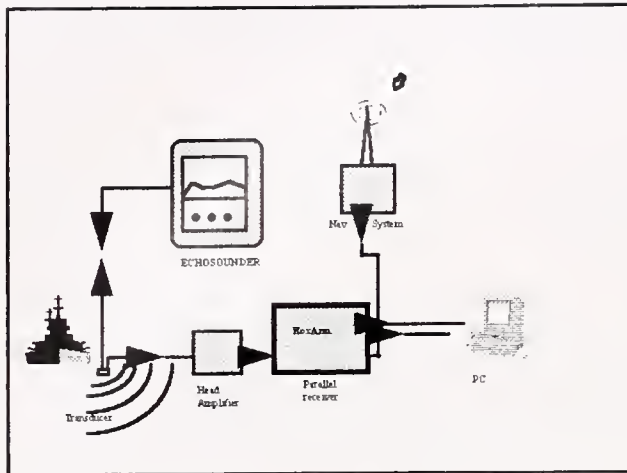


Figure 1. Configuration of a seabed classification system using *RoxAnn*.

longitudinal sound in the seabed; therefore, the strength of these reflections can be thought of as a measure of hardness of the seabed (modified by the roughness of the seabed). The double reflection produces a squared effect on the amplitude of the wave returning to the transducer. Isolation is needed to eliminate the element of hardness already being calculated within the second echo calculation and also to reduce the effect of the sub-bottom reverberation and concentrate on the part of the echo most affected by the roughness of the seabed.

To present information derived from these echoes in a usable manner, it is essential to convert these echoes to a numeric format. Labels E1 and E2 are assigned as indices to indicate the roughness and hardness derived from the first and second echoes. The first index, designated as E1, is derived from the isolated oblique back reflection of the first echo indicating roughness. The second index, designated as E2, is derived from a combination of both amplitude and length of the second echo indicating hardness. E1 and E2, together with depth measurement, are transmitted by a serial link on an RS232 data string to a computer where the data are combined with geographic data from a positioning system and stored.

CONVERTING THE ECHOES TO NUMBERS

To present information derived from these echoes in a usable manner, it is essential to convert these echoes to a numeric format. Labels E1 and E2 are assigned as indices to indicate the roughness and hardness derived from the first and second echoes respectively.

Converting these indices into a numeric format allows the in-

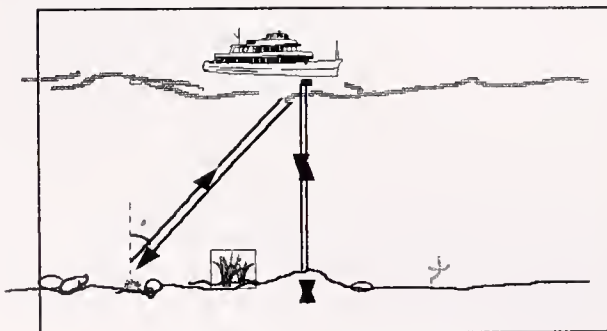


Figure 2. First echo return.

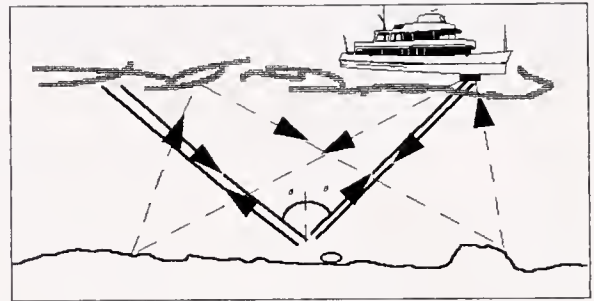


Figure 3. Second echo return.

formation to be displayed on a Cartesian graph. The first index, designated as E1, derived from the isolated oblique back-reflection of the first echo indicating roughness, is plotted on the y-axis. The second index, designated as E2, derived from a combination of both amplitude and length of the second echo indicating hardness, is plotted on the x-axis.

Within both calculations, the use of time varying gain (amplification) circuitry in the electronic receiver eliminates the effect of depth. Within the first echo, it is possible to measure the angle of incidence at which the sub-bottom reverberations will be insignificant. This is dependent upon the depth of water and is used within the calculations of the second echo number. Sub-bottom reverberations become insignificant at a particular angle of incidence.

DISPLAYING THE NUMBERS E1 AND E2

The most obvious method to display two numbers derived independently from seabed measurement is on a Cartesian graph (Fig. 4). This has been called the *RoxAnn* Square. It is possible for a user of *RoxAnn* to create a range of E1 and E2 values that correspond to seabed types. Every seabed material has a particular signature that is a combination of roughness and hardness. Unique from every other type, the *RoxAnn* system discriminates between each selected seabed material type by measurement of signatures. Such common seabed's as sand, mud, rock, and gravel have signatures that can be recognized as having varying degrees of hardness and roughness.

The numbers E1 and E2, generated by *RoxAnn* are transmitted by a serial link with depth measurement on an RS232 data string to a computer where the data can be displayed and stored. The seabed and depth information are first combined with geographic

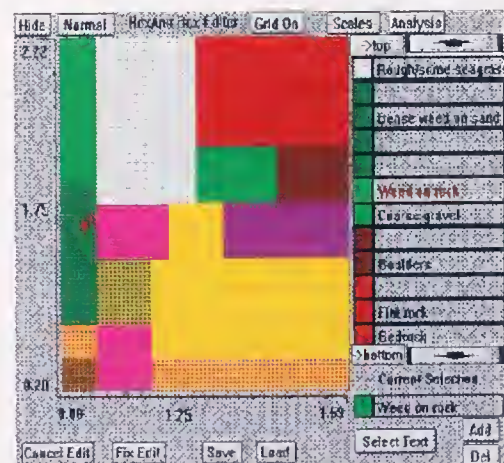


Figure 4. The *RoxAnn* Square.

data from a positioning system and stored. The values E1 and E2 are then processed through the color-coded "look-up table" *RoxAnn* Square within the computer software, where assignment of color to the signatures recorded by *RoxAnn* allow coloring of each geographical position where a *RoxAnn* signature has been generated. A number of navigation and logging software packages display the seabed material data provided by *RoxAnn*, many of which can reprocess data with a high degree of flexibility for the user. The data are in a format suitable for exportation to Geographic Information Systems (GIS) programs. Figure 5 shows a typical computer screen with *RoxAnn* data color-coding the track line of the vessel.

APPLICATIONS OF ROXANN

Classification of seabed sediments and biological communities have now become a recognized requirement for offshore environmental assessments. For example, the extent to which oil or drilling muds with cuttings distribute on the seabed can be observed and thus enable fast and efficient clean-up. Fisheries resource assessments require precise information of the seabed, not only for

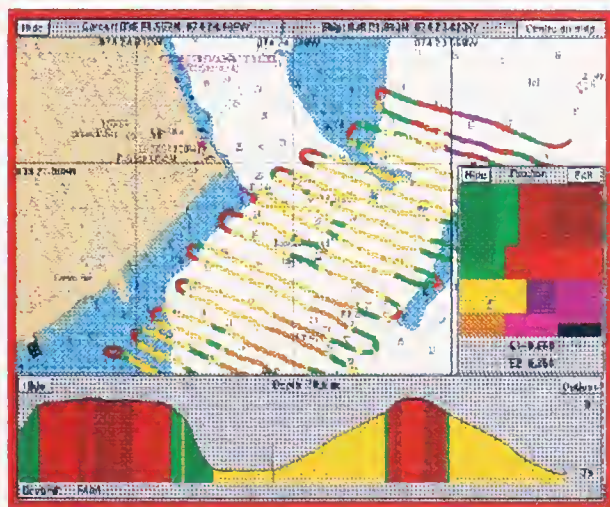


Figure 5. Typical *RoxAnn* two-dimensional display.

RoxAnn - Seabed Classification

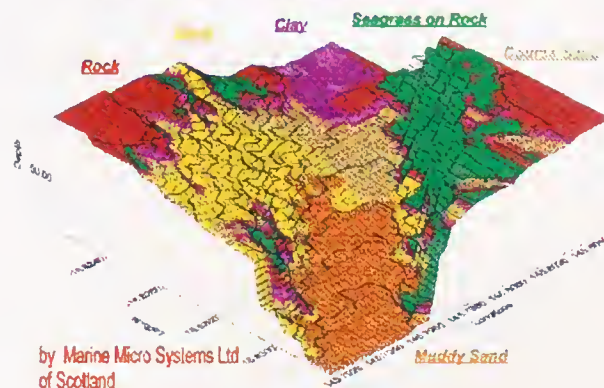


Figure 6. Three-dimensional *RoxAnn* data.

shellfish and demersal fish but also for preferred spawning grounds of pelagic species. Various biologic features, including *Zostera marina*; *Laminaria saccharina*; and *Mytilus edulis*, must also to be detected. Figure 6 shows the capabilities of the system to display seabed information in three dimensions.

CONCLUSIONS

RoxAnn equipment provides a cost-effective means of comprehensive, repeatable, and totally electronic means of natural resource data collection. It allows the user to collect data, process it, and import it into a geographic information system. The ability to produce standardized, comparative type classifications is possible through the combination of the *RoxAnn* and improved software. This seabed classification capability coupled with the Global Positioning System/Differential (GPS/DGPS) provides comparable databases over wide areas that are necessary for long-term monitoring and management needs and provides scientists with a new and powerful tool for multidisciplinary research. During the last decade, *RoxAnn* has proved its value to a wide range of marine and estuarine applications, particularly in fisheries' management, oil spill, natural resources management, and environmental monitoring. (*RoxAnn* is a Registered Trademark of Marine Micro Systems Ltd.)

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MULTIBEAM SONAR: POTENTIAL APPLICATIONS FOR FISHERIES RESEARCH

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ABSTRACT At the core of new ocean mapping technologies is the development of multibeam sonar systems that use beam-forming techniques to insonify large swaths of the seafloor while producing high resolution bathymetry and seafloor imagery. Although the qualitative picture of a distribution of seafloor types is very useful for many applications (including fisheries research), efforts are underway to extract more quantitative seafloor property information from the sonar record. The objective of this work is to provide a robust approach to the remote classification of seafloor types. To facilitate such, several software tools have been developed that allow for the exploration of sonar data in both geographic and bivariate space.

KEY WORDS: Multibeam sonar, bathymetric mapping, seafloor characterization

Over the past few decades, revolutionary changes have taken place in our ability to map and visualize the ocean floor. These changes, brought about by the concurrent rapid advancement of sonar technology, positioning and orientation technology, computer hardware, databases, signal processing and visualization techniques, are beginning to result in detailed depictions of large pieces of the seafloor that are, in many ways, analogous to airborne or satellite-derived images of Earth's surface. At the core of these new technologies is the development of multibeam sonar systems that use beam-forming techniques to insonify large swaths of the seafloor while producing high-resolution (both lateral and vertical) bathymetry and seafloor imagery (backscatter). The Ocean Mapping Group of the University of New Brunswick has been pursuing research and developing tools related to multibeam sonar mapping for a number of years (Hughes Clarke et al. 1996). Although this work has been directed, for the most part, at hydrographic and geologic problems, many of the tools and approaches developed are equally useful for fisheries research.

The extremely high data rates associated with multibeam sonars (as much as gigabytes per hour) present a range of data-processing challenges. The Ocean Mapping Group has developed a full suite of software tools for the real-time and near-real-time display, editing, and visualization of multibeam sonar data that can produce near-finished maps and 3-D images onboard the research vessel. These tools have been used in a number of surveys, including a 1,000 km² area off Eureka, California, a 3,000 km² off New Jersey, and the Stellwagen National Marine Sanctuary. In each case, the combination of detailed bathymetry and sonar imagery provide quantitative depth information and a qualitative description of the spatial distribution of seafloor materials and textures (e.g., rocky areas, sands, gravels).

Although the qualitative picture of the distribution of seafloor types is a very useful tool for a number of applications (including fisheries research), efforts are currently under way to attempt to extract more quantitative seafloor property information from the sonar record. These efforts include the analysis of the characteristics of the vertically incident acoustic waveforms as well as evaluation of the angular dependence of backscatter (Fig. 1). To facilitate this research, several interactive software tools have been developed that allow for the simultaneous exploration of sonar

data in both geographic and bivariate space (Fig. 2). The ultimate objective of this work is to provide a robust approach to the remote classification of seafloor type (Dijkstra and Mayer 1996).

The Ocean Mapping Group has also developed a suite of interactive 3-D data exploration tools to facilitate the interpretation of these complex datasets. A 6-degree-of-freedom mouse (Bat) allows for interaction with massive (10s to 100s of megabytes) datasets with simple hand movements and exploration in a natural and intuitive fashion. Sonar backscatter data, derived or measured sediment properties, or a range of other parameters can be draped over the 3-D bathymetry and interactively explored (Figs. 3, 4). Datapoints can be selected in 3-D for position, depth, or other attributes, and measurements can be made in the 3-D space (3-D GIS, Figs. 5, 6); the 3-D scene can be viewed in true stereo with special glasses (Mayer et al. 1997b). We have recently been utilizing these tools for the real-time visualization of midwater targets, including schools of fish. These efforts are, in the short-term, directed toward delineating the lateral extent and dynamics of fish schools (Mayer et al. 1997a). In particular, we are using the wide swath-widths available with multibeam sonars to address the issue of vessel avoidance (Fig. 7). Future work will concentrate on target density determinations and, if properly calibrated, biomass calculations.

Properly processed multibeam sonar data provide the ability to extract quantitative information about both the shape and character of the seafloor, to manipulate and overlay multiple geographically referenced datasets to establish derivative relationships, and to visualize and interact with these massive datasets in a natural and intuitive manner. This ability to characterize bottom shape and potentially bottom type remotely and rapidly should have important ramifications on several aspects of fisheries research.

One of the most obvious applications of multibeam mapping is in support of habitat studies for invertebrate species. The data provided by multibeam sonars is ideal for understanding the distribution and nature of ground fish habitats and their relationship to commercial fisheries (and other species associations). By taking advantage of the detailed information on seafloor shape and type provided by these systems, fisheries ecologist can begin to relate habitat to ecological patterns (much like terrestrial ecologists have been able to do). Although being able to collect and process data

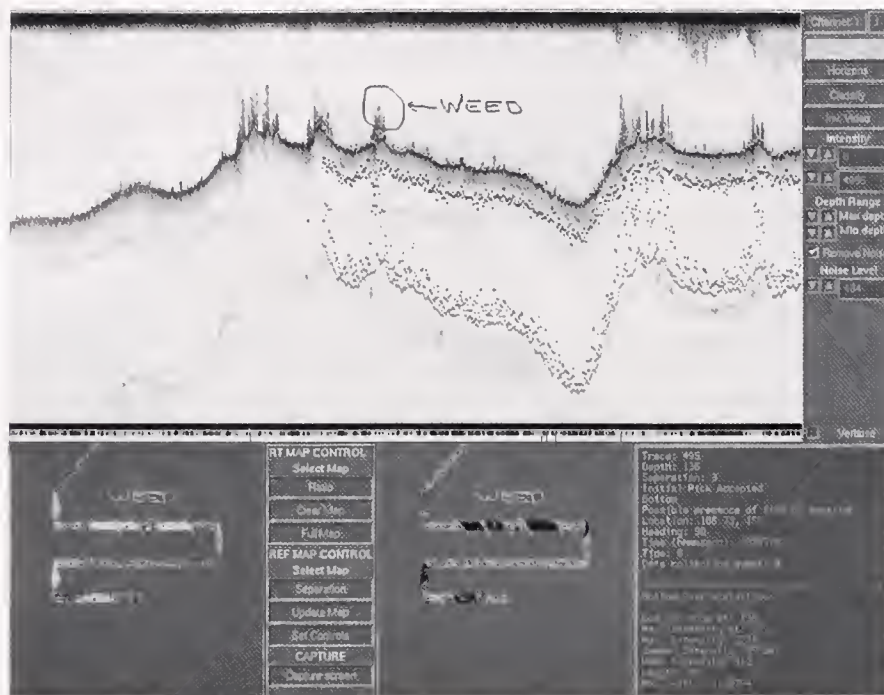


Figure 1. Real-time sediment classification from vertical incidence sounders. Upper window is continuous scrolling display of profiler data (in this case from a 210 kHz Navitronics sweep system collecting data in an intertidal area of St. John Harbour, New Brunswick). Horizon picks are displayed as color-coded dots beginning about one-third along the window. The two lower graphics windows can be set to display a range of parameters including individual waveforms with picks or various characteristics or statistics of the waveforms in a geographic context. The text window in the lower right reports the statistics and details of the currently processed trace. In this example, the separation between the leading edge of the trace and the peak amplitude is displayed as a gray-scale intensity plot in geographic space. The resulting plots (plotted here with large separation as dark in left window and light in right window) produce a clear and accurate portrayal of the distribution of seaweed in the area.

in near-real-time can be of importance in survey and sampling planning, it is also important to note that, for the most part, the seafloor is relatively slowly changing, and, thus, any survey data collected can be archived and used as a base for comparison with retrospective data and for future work. A project of this sort is being planned for the New Jersey margin where a large historical database of landing records will be superimposed on, and compared with, the newly collected detailed images of the seafloor. In those areas where there are active changes in the seafloor (e.g., areas of sediment transport or bedform migration), repeat surveys can rapidly document and quantify the degree of mobility of the seafloor.

The increased bathymetric detail produced by multibeam sonar may also prove to play a critical role in providing boundary conditions for the ever increasingly high-resolution coupled ocean circulation models that are currently being used to enhance our understanding of biological and fisheries processes. Most models now use the standardly available ETOPO-5 bathymetric database that is gridded at 5 mile resolution. Although this spacing is coincident with the $1/2$ degree resolution of some GCMs, higher bathymetric resolution will be needed as higher-resolution regional models are developed. The growing collection of multibeam data is an ideal source for the needed bathymetric data, and, in critical regions, new multibeam surveys can be carried out.

Finally, multibeam bathymetry and imagery provide ideal means of examining the impact of human activities on the seafloor and on the fisheries. Many modern multibeam sonars can easily discern trawl marks and debris or dredge spoils dumped on the

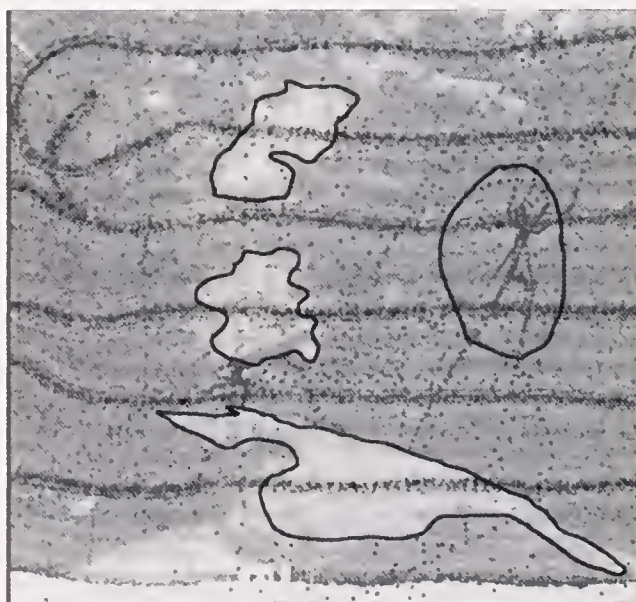


Figure 2. Classification training tool (LASSOO). Superposition of physical or acoustic property data on top of sidescan sonar imagery. Here *RoxAnn* data (E1/E2) are superimposed on 95-kHz backscatter data from EM-1000 multibeam sonar. The EM-1000 imagery clearly defines the dredge-dump sites (two areas in the central region) as well as regions of gravel, bedrock, and background substrate. LASSOO allows these regions to be selected and assigned classes.

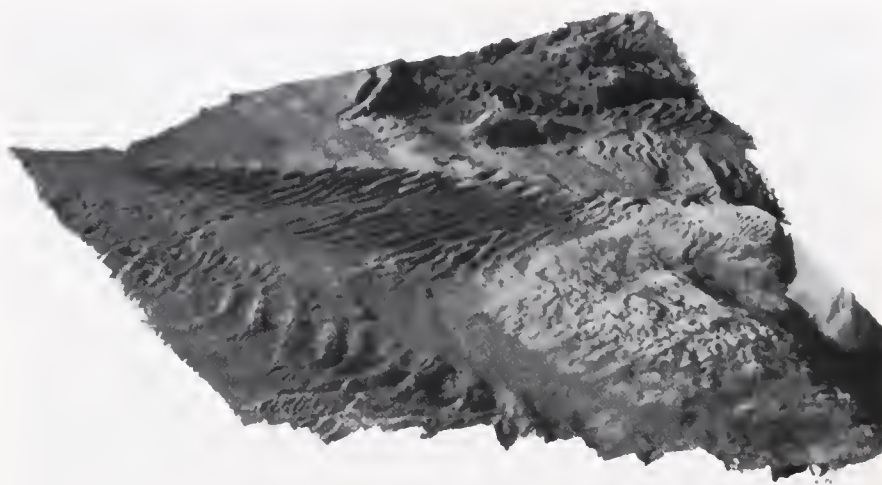


Figure 3. 3-D visualization of multibeam sonar (EM-1000) dataset collected around Alcatraz Island in San Francisco Bay. Our software tools allow datasets such as this to be color coded (by any attribute) and interactively explored in 3-D (including stereo).

seafloor. Multibeam surveys can quickly pinpoint the location and spatial distribution of human activities and help to focus the subsequent sampling and monitoring needed to assess the impact of this activity on the fisheries. Repeat surveys provide the opportunity to monitor the fate of feature (i.e., how long do trawl marks last, or how are dredge spoils spatially distributed?) and once again allow sampling and monitoring programs to be designed around a

clear knowledge of what is on the seafloor rather than a blind guess.

Multibeam data is continuously being collected worldwide, for purposes other than fisheries research (geologic, hydrographic, cable and pipeline routes, and military surveys). Much of these data are available to the fisheries research community, but they must seek it out and learn how to take full advantage of it.

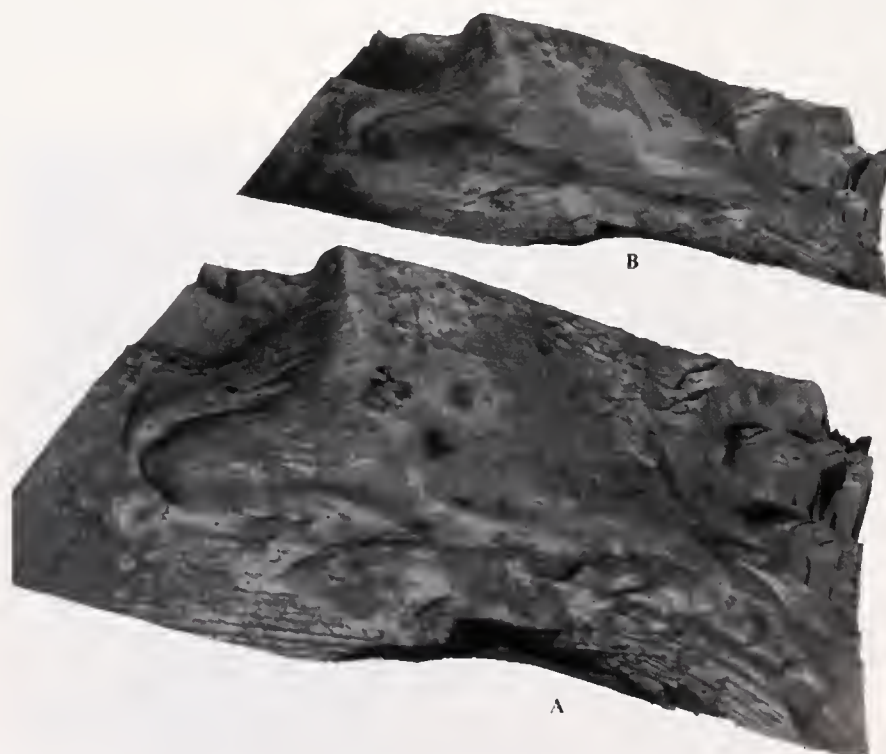


Figure 4. 3-D visualization of two of the 12 parameters extracted from the angular response of backscatter data from a 30×27 km area of Stellwagen Bank National Marine Sanctuary, Mass. Each is draped over bathymetry and displayed (in original) with color coding. Lower figure (A) represents slope of segment of backscatter curve between 75° and 30° grazing; upper figure (B) represents mean backscatter between 50° and 40° grazing. Parameterization of the backscatter curve may provide important insight into the distribution of seafloor properties; visualization in this mode shows the direct relationship between these parameters and the seafloor morphology.

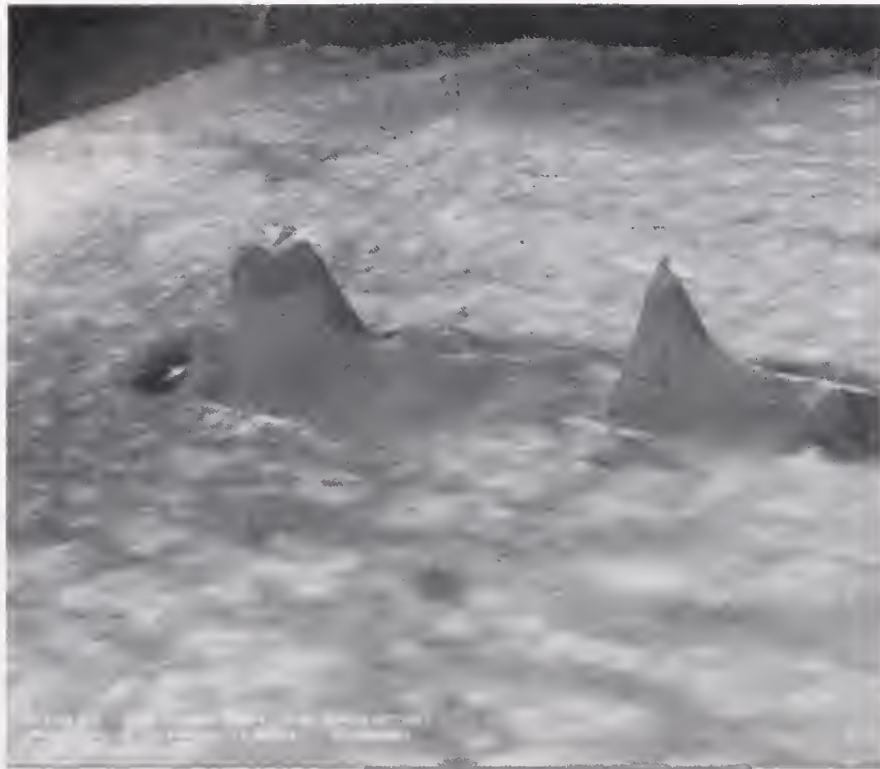


Figure 5. Single frame from interactive flight through EM-1,000 multibeam bathymetry collected over 150-m long wreck of freighter *British Freedom* outside of Halifax Harbour demonstrating ability to pick, make measurements, and interrogate points in 3-D—picked points are marked with cross-hairs. Attributes of latest point are displayed in lower left of screen and can be saved to file for later reference.



Figure 6. 2-D component of pipeline and cable-route planning tool. Multibeam sonar imagery is draped over bathymetry providing simultaneous depth and seafloor property information for route planner—prospective routes can be interactively explored with cross sections, gradients, and segment lengths displayed. The example above is from an application where real-time navigational information allowed the progress of the cable-laying vessel and the plow (cross hairs) to be monitored within the context of previously collected multibeam sonar data.



Figure 7. Snapshot of real-time 3-D visualization of herring school.

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PHYTOPLANKTON BLOOMS AND REMOTE SENSING: WHAT IS THE POTENTIAL FOR EARLY WARNING

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ABSTRACT Phytoplankton blooms are closely coupled to physical processes. Increased access to wind, sea surface temperature, and ocean color datasets aids in retrospective analyses of bloom events and provides vital linkages of physical and biological processes. From such analyses, conceptual models of bloom initiation, transport, maintenance, and dissipation may help identify the timing of bloom initiation and define environmental conditions conducive to growth. The advent of on-line, real-time environmental data, new ocean color sensors, and hyperspectral scanners may allow enough predictive capability so that harmful or toxic bloom conditions can be detected, research efforts focused, and reliable information made available, before effects of these blooms are manifest in near shore areas.

KEY WORDS: plankton blooms, remote sensing, detection

INTRODUCTION

The physical, physiological, and behavioral characteristics of phytoplankton bloom species affect their detection by remote sensing methods. Detection is based on the differential absorption and backscatter of irradiance; certain species are more amenable to detection because of the reflectance characteristics of the cells themselves (packaging) (Millie and Schofield 1995). Surface blooms of coccolithophores with external calcium carbonate plates, diatoms with silica frustules or spines, and cyanobacteria with highly reflective gas vacuoles form extensive patches that are highly reflective and readily detected on visible-band images collected by the CZCS (Coastal Zone Color Scanner) on Nimbus-7, the Landsat MSS (Multispectral Scanner) series and the AVHRR (Advanced Very High-Resolution Radiometer) on NOAA polar orbiting weather satellites (Ackelson et al. 1988, Balch et al. 1991, Kahru et al. 1994, Subramaniam and Carpenter 1994). Monospecific blooms of large, phototactically positive cells with numerous chloroplasts provide a strong signal and may be detected by ocean color sensors at cell concentrations 10- to 100-fold less than are required for visual detection of "discolored water" (Tester et al. 1998). Such blooms may also absorb enough energy to increase the water temperature by >1°C and be seen as a warm "patch" in thermal imagery (Kahru et al. 1993). Development of phytoplankton group-specific algorithms has targeted AVHRR imagery (Tassan 1993a), but this has been most successful in defined situations employing high-resolution multispectral scanners and detailed pigment analyses (Millie et al. 1992, Sakshaug et al. 1991).

Equally important to detection of phytoplankton blooms by remote sensing is the spectral quality, thermal signature, and hydrographic features of the waters surrounding a bloom. Frequently blooms are found along frontal zones, and these hydrographic features may be coherent over scales of 10^2 – 10^3 km. The physical and biological factors affecting bloom dimensions are critical, because resolution of patches <10–100 km² is not generally possible. Major current systems (Gulf Stream, Kuroshio Current) are frequently implicated in the transport of blooms, and these currents can be tracked most simply and reliably using thermal AVHRR

imagery (Satsuki et al. 1989, Tester et al. 1991). Blooms occurring in optically clear, offshore waters are more easily detected and interpreted, however, algorithm corrections for AVHRR, CZCS, and Landsat TM data from turbid systems have been developed to make bloom definition in coastal or estuarine environments possible (Stumpf and Tyler 1988, Tyler and Stumpf 1989, Eckstrand 1992).

A conceptual model of species specific bloom dynamics is valuable in understanding conditions conducive to bloom initiation and development. If one or several of the environmental cues necessary for bloom initiation, development, or transport are detectable via remote sensing techniques, they can serve as important signals for early warning (Tester and Steidinger 1997). Local bloom development may be characterized by differential stratification of the water column, weak tidal currents, and low wind speeds. Transport of blooms is associated with strengthening and persistence of wind conditions, upwelling–downwelling events, or entrainment into fronts or currents. Bloom dispersal results from mixing and flow divergence. For more specific information about harmful algal blooms in U.S. waters, their ecology and oceanography (ECOHAB 1995) see the following web site: <http://habserv1.whoi.edu/hab/nationplan/ECOHAB/ECOHABhtml.html>.

Sensors

AVHRR

One of the most popular instruments for the oceanographic community is the Advanced Very High-Resolution Radiometer (AVHRR) that provides sea surface temperature (SST) imagery. Satellite SSTs are made by converting the radiance measured in the infrared channels to brightness temperatures. This sensor on-board polar orbiting environmental satellites (POES), better known as weather satellites, has been observing Earth since the first launch of TIROS 1 in 1960. The current series of satellites began with the launch of TIROS-N (Advanced Television Infrared Observation Satellite) in 1978. The United States operates two satellites simultaneously, with each of these circling the globe 14 times

per day. The POES satellites continuously collect information from a 1,700-mile wide strip of Earth below, progressing westward on successive orbits and provide two complete pictures of Earth every day from each satellite. Continuous synoptic coverage appropriate to feature tracking with a pixel resolution of 1.0–1.4 km is provided.

Ocean Color

Coastal Zone Color Scanner flown on Nimbus-7 from November 1978 to mid-1986 was specifically designed to observe ocean color and make quantitative measurements of oceanic radiance (443, 520, 550, 670, 750 nm). Phytoplankton pigment (chlorophyll) concentration in surface waters can be related to ocean color either empirically, using oceanic observations, or theoretically, using the theory of radiative transfer in sunlit waters (Stewart 1985). Consequently the CZCS data have been used extensively to estimate phytoplankton biomass and primary productivity over large regions (Gregg and Walsh 1992, Behrenfeld and Falkowski 1997). It is one of the most valuable and extensively used datasets available to the oceanographic community; the CZCS images can be viewed and data ordered via the following web site: http://daac.gsfc.nasa.gov/daac-bin/db_czcsbns

After more than a decade without ocean color imagery, the Ocean Color and Temperature Sensor was launched in 1997 but, unfortunately, was operational for only a few months before debris struck its solar panel and rendered it inoperative. The long-awaited NASA's SeaWiFS (Sea-Viewing Wide Field-of-View) sensor was launched by NASA on 1 August 1997 and should prove to be even more sensitive than its predecessor. Check out the latest information regarding SeaWiFS at: <http://seawifs.gsfc.nasa.gov/SEAWIFS.html>

Other

LANDSAT (land-observing satellites) were designed to view scenes with a wide range of brightness, so their multispectral scanners are poorly suited to observe the subtle variations in the hue of the oceans that indicate the distribution of riverborne sediments and phytoplankton, especially over shallow bottoms (Stewart 1985). Despite this, its high-resolution <80 m has tempted some researchers to examine thematic mapper images for information on thermal effects, suspended sediment concentrations, and chlorophyll *a* (Garcia and Robinson 1991, Tassan 1993b, Daby 1994).

RADAR SAT

Synthetic aperture radar (SAR) satellite Earth observation techniques represent a new tool to detect ocean features independent of light and weather conditions (see Staples et al. 1997). RADAR SAT-1 launched 4 November 1995 has variable resolution (depending upon beam mode, incidence angle) from 8 × 8 m to 100 × 100 m and may prove most valuable when other sensors are limited by ambient weather conditions.

LIDAR

The multispectral scanner, a multiwavelength instrument with >250 band widths can be flown in a conventional aircraft or, in a simpler version, suspended from a buoy to characterize the spectral quality of the water. Species-specific algorithms are particularly useful when toxic species (flagellates) distinguished from nontoxic

(diatoms) (Sakshaug et al. 1991) and the results combined with pigment analyses for confirmation (Millie et al. 1992).

Remote Sensing Detection of Harmful Algal Blooms

Sea surface temperature (AVHRR) imagery has been used to study the bloom dynamics of *Alexandrium tamarense* and the onset of paralytic shellfish poisoning in the southwest Gulf of Maine (see Keafer and Anderson 1993). Comparative imagery from 1989 to 1991 detected a warm coastal current that formed from spring runoff and served to transport *A. tamarense* cells south along the New England coast. Coastal upwelling was detected in 2 of the 3 years of study and moved the warm, buoyant plume containing *A. tamarense* cells offshore and away from nearshore shellfish beds. The thermal imagery was valuable in helping understand the short-term oceanographic processes responsible for the development and behavior of the plume and the subsequent distribution of *A. tamarense* cells. The thermal features associated with spring runoff, a low density coastal plume, and regional upwelling have predictive power, because they can be monitored to detect conditions conducive to *A. tamarense* bloom initiation and transport.

Gymnodinium breve blooms are common along the west coast of Florida and have been recorded there in 21 of the last 22 years. The onset of these blooms in association with the onshore movement of the (Gulf) Loop Current has been noted (Tester and Steidinger 1997). However, this had never been quite so dramatically demonstrated until February 1996, when a thermal feature (AVHRR 14 February 1996; see Stumpf et al. 1998) transported *G. breve* cells into Florida coastal waters where 158 endangered manatees died of exposure to brevetoxins between 1 March and 1 May 1996 (Landsberg and Steidinger 1998).

Another red tide bloom event was the product of transport of *G. breve* cells by the Gulf Stream (see Tester et al. 1991, Tester and Steidinger 1997). About 30 days after a red tide bloom near Charlotte Harbor–Sarasota, Florida, satellite images of sea-surface temperature (AVHRR) substantiated the shoreward movement of a filament of Gulf Stream water onto the narrow continental shelf between Cape Hatteras and Cape Lookout, North Carolina. This filament was a source of *G. breve* cells, and it remained in near-shore waters and was identifiable in satellite images for >19 days.

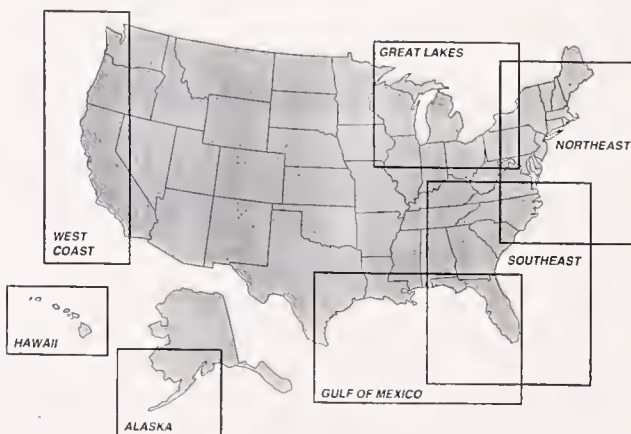


Figure 1. NOAA CoastWatch regional locations for production and near-real-time distribution of AVHRR (advanced very high-resolution radiometer) sea surface temperature images for U.S. coastal researchers and decision makers.

This was the first recorded occurrence of *G. breve* north of Florida and represented a range extension of >800 km. During the bloom that followed for the next 4 to 6 months, shellfish beds were closed, causing an estimated \$25 million loss to fisheries and tourism.

NOAA's polar orbiting satellites were able to detect an ocean thermal feature associated with the event. These sea surface temperature (AVHRR) images provided a means for understanding the oceanographic mechanisms responsible for the occurrence and distribution of these toxic phytoplankton. The imagery has since been used for a variety of marine research, management, and educational purposes and is now available for all U.S. coastal waters, includ-

ing those of Alaska, Hawaii, and the Great Lakes (Fig. 1). Coast-Watch web address is: <http://psbgsi1.nesdis.noaa.gov:8080/PSB/EPS/CW/coastwatch.html>

Furthermore, the NOAA Center for Coastal Services, Charleston has an experimental project designed as a "proof of concept" that will attempt to forecast *G. breve* blooms for the west Florida shelf using a combination of satellite imagery and meteorological data (J. Brock, pers. commun.). If the project is implemented, it will be a major milestone in the efforts to forewarn coastal residents, researchers, and marine resource managers about the occurrence and distribution of harmful algal blooms (Boesch et al. 1996).

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GEOGRAPHIC INFORMATION SYSTEMS (GIS) AND KRIGING: ANALYSIS OF THE SPATIAL AND TEMPORAL DISTRIBUTIONS OF THE OYSTER PATHOGEN *PERKINSUS MARINUS* IN A DEVELOPED AND AN UNDEVELOPED ESTUARY

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ABSTRACT Infection and prevalence of *Perkinsus marinus* (Dermo), a common protozoan pathogen of the oyster (*Crassostrea virginica*), are commonly correlated with temperature and salinity. However, these factors do not account for all infection variability. Other factors such as watershed dynamics or anthropogenic disturbances may play a role. Geographic Information System (GIS) technology applying land use and anthropogenic activity was used to document Dermo infections in two South Carolina coastal estuaries. Kriging analysis was used to calculate disease prevalence from sample sites to the estuary as a whole. Results indicate that land use patterns may affect the distribution of the disease.

KEY WORDS: GIS, kriging, *Perkinsus marinus*, *Crassostrea virginica*, oyster, disease

INTRODUCTION

Spatial data from estuarine area are often collected from discrete points. Therefore, the ability to detect or resolve spatial and temporal patterns is dependent upon the density of sampling points. A compounding problem is the highly variable and complex nature of estuarine systems. The use of Geographic Information Systems (GIS) combined with the geostatistical analysis known as kriging provides unique opportunities to study the spatial and temporal patterns of sampled data. Kriged data overcome the difficulty of acquiring enough datapoints to resolve spatial patterns. In conjunction with GIS, this method allows for the analysis of multiple variables, yielding an integrated spatial model of the study site.

Kriging is an advanced interpolation method that generates a continuous distribution of values from a set of discrete sample data (Burrough 1987, Little et al. 1997). The end product of this process is a continuous surface or map of predicted values based upon the observed values. The predicted values are derived from the weighted average of the sample values dependent upon the distances between the sample sites and target locations (Burrough 1987, Little et al. 1997). Predicted value locations are obtained from a grid representing the study area, resulting in a map, which is georeferenced to the study site. The data layer is then integrated into a GIS, allowing for analyses with such ancillary data as land use, land cover, and environmental variables (Porter et al. 1997). Prediction accuracy is measured by a process of cross validation, which involves the removal of a point from the observed sample, and then deriving a new data layer of predicted values. An over-all performance measure is then calculated, the prediction sum of squares or PRESS statistic:

$$\text{PRESS} = \sum_{i=1}^n [Z_i - \hat{Z}_{(-i)}]^2$$

where Z_i denotes the i th sample value, and $\hat{Z}_{(-i)}$ denotes the prediction for this value based on the number of points of the new set ($n - 1$).

To illustrate the technique of kriging in a GIS and its application to invertebrate fisheries, this paper describes our ongoing efforts to identify factors that influence the spatial and temporal patterns of *Perkinsus marinus* (Mackin, Owen, and Collier), a common protozoan pathogen of the eastern oyster *Crassostrea virginica* (Gmelin). Prevalence and infection intensity of this parasite is commonly correlated with temperature and salinity (Craig et al. 1989, Andrews 1996, Bureson and Calvo 1996); however, much of the observed variability, particularly the spatial variability, remains unexplained (Wilson et al. 1990, Calvo et al. 1996, Soniat 1996). On a relatively large regional scale, land-use patterns (e.g., agricultural, industrial, urban) and such related contaminants as polynuclear aromatic hydrocarbons have been correlated with the prevalence and intensity of *P. marinus* (Craig et al. 1989, Wilson et al. 1990). To help identify relationships between land-use patterns and their effects on invertebrate fisheries, this paper reports on the spatial and temporal distributions of *P. marinus* infection levels of *C. virginica*.

METHODS

Preliminary spatial data were collected at North Inlet (an undeveloped estuary) and Murrells Inlet (a developed estuary). Both estuaries are shallow, tidally dominated systems that are located about 20 miles apart on the northern coast of South Carolina (Fig. 1). This preliminary data, which included the physical geography and the distribution of dominant habitats as well as the surrounding land-use patterns, were entered into a GIS to produce a descriptive map of each site.

Ten oysters were assayed for prevalence and infection intensity of *P. marinus* (Ray 1952) from a total of 30 oyster reefs at Murrells

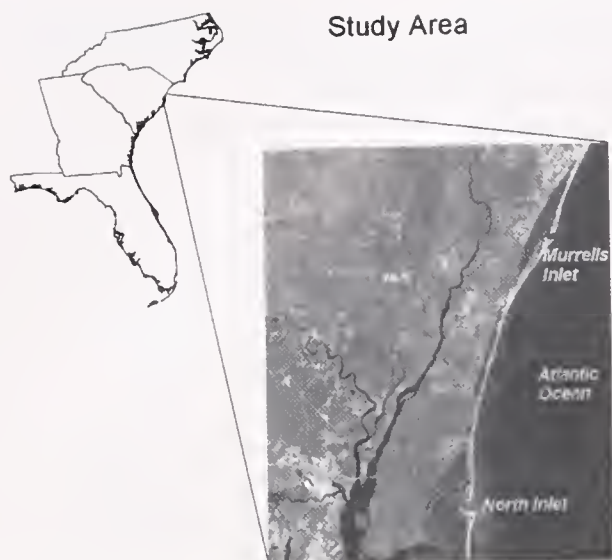


Figure 1. Map of South Carolina coast showing proximity of Murrells Inlet and North Inlet.

Inlet over the course of a week in September 1996 and again in December 1996. At North Inlet, 35 reefs were similarly sampled in September and 37 in December. Infection intensity was ranked on a scale of 0 (no infection) to 5 (extreme infection) for each oyster and an average determined for all oysters from each reef. This average is termed the "weighted prevalence" (wp).

Weighted prevalence for each oyster reef site was the input vector for the sample values used in the kriging analyses. These data were combined with the Universal Transverse Mercator (UTM) coordinates of each sample site as determined from a Global Positioning System (GPS) survey. Additional inputs included a grid based on the lower left and upper right quadrants of the study areas and the grid cell size in UTM coordinates. Kriging analyses were performed on a Sun Sparc station (10[®] using Splus[®] functions (Rao 1992, Little et al. 1997). GIS analyses were performed on a Sun Sparc station 10[®] using ArcInfo[®] and a PC using ArcView[®].

To illustrate our spatial analytic techniques better, data from Murrells Inlet, September 1996 is used as an overview. After each kriging analysis, the predicted variable set, containing a predicted value for each grid cell, is imported as an ASCII file into ArcInfo[®] and converted into a point file (Fig. 2). This is a data layer of the discrete predicted values. Using GIS techniques, a continuous surface of predicted values is then generated from the point file (Fig. 3). A georeferenced data layer that represents the Murrells Inlet creek system is then used as a mask to produce a continuous surface of infection intensity specific to the creek system (Fig. 4). This data layer is then integrated with other data layers of the inlet, describing land-cover and land-use patterns, to produce a continuous surface model of predicted infection intensity for *P. marinus* throughout the inlet (Fig. 5).

RESULTS

Data from Murrells Inlet show alternating patterns of mild to low infection levels throughout the estuary during September 1996

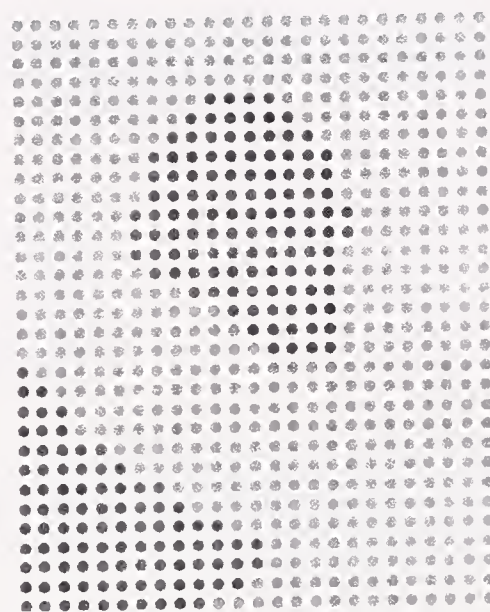


Figure 2. Point file of predicted infection intensity for *P. marinus* produced in ArcInfo[®] after kriging in the software package Splus[®].

(Fig. 5). These infection intensities are considered nonlethal. In contrast, December 1996 data show moderate to heavy infections in most creeks, except those buffered by a state park in the southwestern extent of the estuary (Fig. 6). The over-all increase in infection intensities from September to December throughout the inlet may reflect seasonal patterns of the disease (Crosby and Roberts 1990, Bureson and Calvo 1996). However, infection intensity remained relatively low between the September and December sampling periods within the state park, while increasing in areas surrounded by development (Figs. 5, 6). The highest intensities were found along a dredged channel (Fig. 6). These data



Figure 3. Continuous surface of predicted infection intensity for *P. marinus* produced from the ArcInfo[®] point file (Fig. 2.).



Figure 4. Continuous surface of predicted infection intensity for *P. marinus* specific to the Murrells Inlet creek system, produced from the continuous surface data layer (Fig. 3), using a mask of the Murrells Inlet creek system.

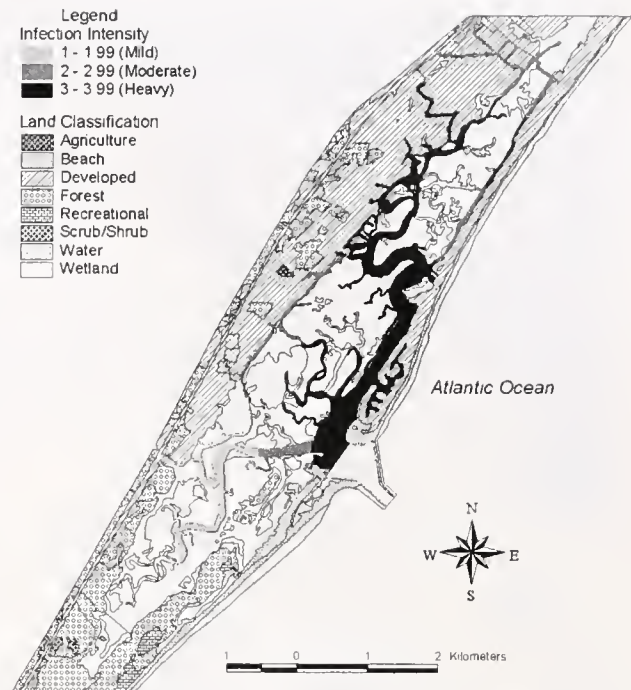


Figure 6. Predicted infection intensity of *P. marinus* in Murrells Inlet, SC, December 1996.

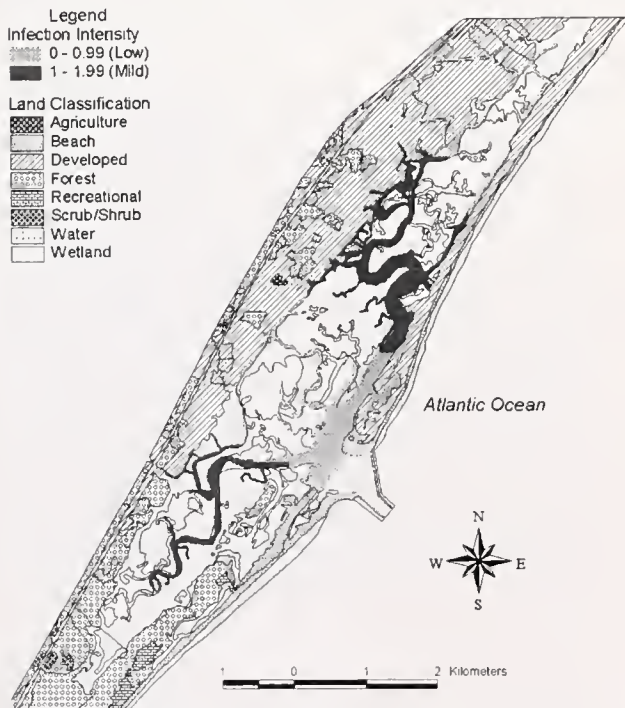


Figure 5. Predicted infection intensity of *P. marinus* in Murrells Inlet, SC, September 1996.

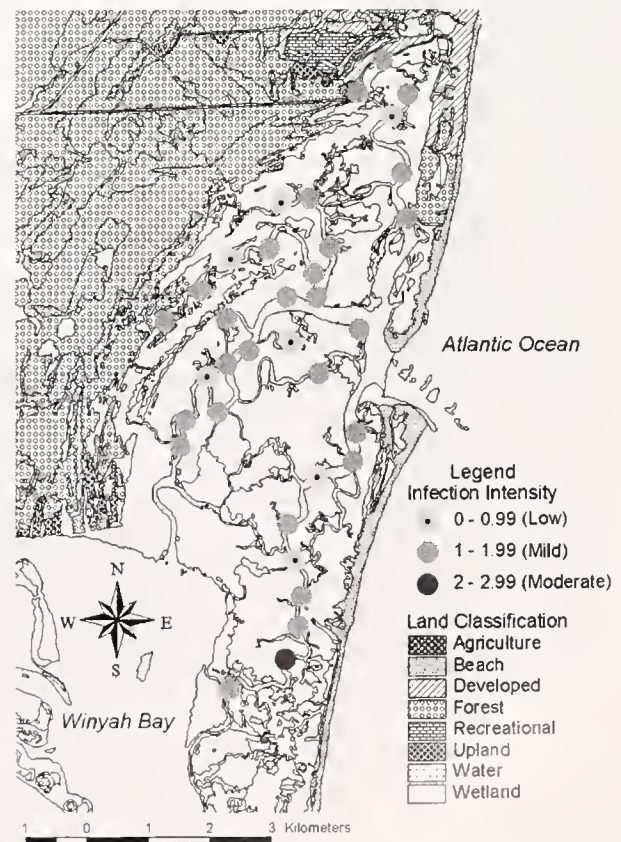


Figure 7. Sampled values of infection intensity *P. marinus* in North Inlet, SC, September 1996.

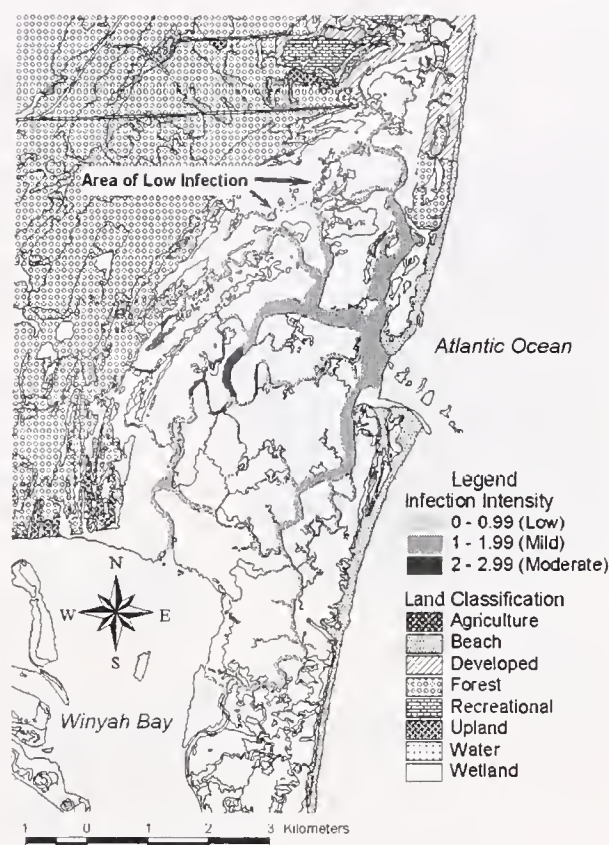


Figure 8. Predicted infection intensity of *P. marinus* in North Inlet, SC, December 1996.

indicate that land-use patterns may be affecting the prevalence and intensity of *P. marinus* infections in *C. virginica* populations within this inlet.

In North Inlet, low infection intensities were observed throughout the estuary in September (Fig. 7). The corresponding kriging analysis was inconclusive because of a lack of any detectable spatial trends in the data. This result indicates that differences in infection intensity among sites were small and more or less random. In contrast to Murrells Inlet, infection intensities remained relatively low during the December 1996 sampling period (Fig. 8). Areas of higher infection intensities corresponded to sites at local salinity nodes demarcating the influence of fresh water from Winyah Bay. Because of local hydrography, these sites are likely to be areas poorly flushed with the outgoing tide relative to the rest of the estuary.

CONCLUSION

Geographic Information Systems combined with kriging results in statistically robust models can facilitate the study of spatial and temporal factors involved in *P. marinus* infections. We believe that continued development of these tools will lead to increased accuracy in monitoring and predicting levels of *P. marinus* infections. More importantly, these tools will help identify natural and anthropogenic factors that control *P. marinus* and its impact on eastern oyster populations. Such insight will lead to the development of better management strategies. GIS and kriging can be similarly applied to other problems of invertebrate fisheries. As coastal resources continue to experience increased development, it is imperative to understand how biological resources respond to coastal development. Some of the possible effects on ecological systems and local economies could be devastating. GIS and kriging are valuable tools that can help identify factors creating problems for invertebrate fisheries, and thus lead to the development of viable solutions or management strategies.

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A SUBMERSIBLE STUDY OF RED KING CRAB AND TANNER CRAB DISTRIBUTION BY HABITAT AND DEPTH

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ABSTRACT The summer distributional patterns of red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) were examined by manned submersible in a southeast Alaska fjord. Six habitats types, including sand–mud, rock, shell, debris, rock wall, and algae, were identified from video tapes. Highest crab densities for both species were in the sand–mud habitat. No crabs were found in the rock wall and algae habitats. Both species displayed a dome-shaped distribution with depth, peaking at 75 m for king crab and 145 m for Tanner crab. Dense aggregations of juvenile king crabs were observed in a cove. No mature crab aggregations were observed.

KEY WORDS: Submersible, king, and Tanner crabs, distribution

Crab pots and trawls typically have been used for crab surveys. These methods are habitat restricted and either attract crabs from unknown distances (pots) or sample expansive areas of the bottom (trawls). They provide poor information on the microscale distribution of the crabs and their distribution by habitat and depth.

Manned submersibles are a relatively new technique for underwater surveys but have an increasing use in the study of benthic invertebrates and demersal fish on a variety of substrata (Pearcy et al. 1989, Tyler and Zibrowius 1991, Krieger 1993, Stein et al. 1992, O'Connell and Carlile 1993, Stevens et al. 1994, Starr et al. 1996, Zhou and Shirley 1996). In this study, we employed the *DELTA* research submersible to investigate the distribution of red king crab (*Paralithodes camtschaticus*) and the Tanner crab (*Chionoecetes bairdi*) by habitat and depth in a southeastern Alaskan fjord and to compare the dispersion patterns of the two species during the summer season.

The study was conducted at Barlow Cove (58°22'N, 134°53'W), southeastern Alaska, from June 18 to 22, 1991. We used a two-man research submersible, the *DELTA*, to conduct transects along the bottom of the cove. The *DELTA* has 19 viewports, one external color video camera with internal monitor, one internal hand-held video camera, one external bulk loaded 35-mm camera, and one internal hand-held 35-mm camera. Transects were made during daylight. All dives were initiated from a randomly selected location at the center of the cove to the shoreline, with the transect oriented perpendicular to the long axis of the cove. The support ship tended the submersible and periodically established the location and transect direction of the submersible. During the transect, the pilot attempted to maintain the submersible at a constant speed and at a constant height above the bottom. Two video cameras continuously recorded the sea floor as the submersible moved along the transects. Additional still photographs were taken, and direct observations were recorded for the identification and analysis of animals and habitat types.

The time, temperature, depth, and the height of the depth transducer of the submersible off the sea floor were automatically recorded every 20 s. The area of camera coverage in one transect is expressed by $A = W * L$, where W is the width of front side (wider side) of the video view field and L is transect length. The number of crabs was counted from the video tapes recorded by the fixed external video camera, and the density was obtained by dividing this number by the area A .

RESULTS AND DISCUSSION

Habitats Identified from Submersible

Six types of substrata were identified from the transect: sand–mud, rock, shell, debris, rock wall, and algae. Rocks were usually cobble-sized. Debris consisted of decaying wood and algae. The algae substratum consisted of living marine plants, such as kelps. A deep, flat, sand–mud substratum along the central cove occupied most of the bottom. This central part of the cove was bordered by steep rock walls on both sides, except in the innermost part of the cove. Bivalve shells formed a substantial percentage of the substrate in some depth zones, usually at the upper and lower edges of the rock wall. On average, the sand–mud substratum comprised 60.2% the sea floor. Cobble, shell, debris, rock wall, and alga accounted for 15.9, 11.6, 3.0, 5.7, and 3.6%, respectively, of the sea floor. Debris was mainly found in the inner cove on the central, flat bottom.

Direct Observations of Red King Crabs and Tanner Crabs

In most cases, red king crabs were observed foraging individually, separated more than 10 meters apart from other conspecifics. Individuals of both the red king crab and Tanner crab did not aggregate, except juvenile red king crabs with carapace length less than 80 mm. The submersible seemed to cause no obvious disturbance to the behavior of adult crabs when one meter or more away from the crabs. Tanner crabs were frequently observed partially buried in the mud, with only the chelipeds and front carapace visible for some buried crabs. In two dives in the evening (around 8:00 PM) in the inner cove, a large number (estimated to be at least several thousand) of juvenile red king crabs were observed in pods. These crabs were approximately 50 to 70 mm in carapace length, and may have been 3 to 4 years of age. They physically contacted each other and were stacked up to several layers deep. Their quick moving and crawling on the top of other crabs indicated that the crabs were separating for foraging at night (Dew 1990) or responding to the submersible. On several other occasions during daytime in the inner cove, red king crabs of similar size as the podding crabs were observed close to each other but were not in physical contact. From the inner cove to the outer cove, the size of red king crabs tended to increase.

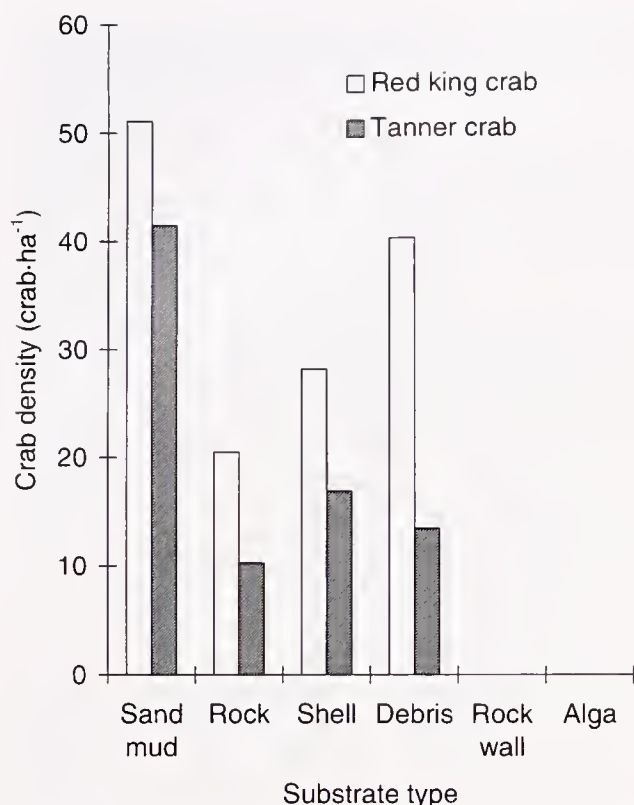


Figure 1. Density of red king crab and Tanner crab by substrata types. No crabs were found on rock wall or algae habitats.

Crab Distribution by Habitat

A significant difference in density existed among the substrata, even if the rock wall and algae habitat, where no crabs were observed, were excluded (G-test, $p < .05$, $df = 3$, for both red king crab and the Tanner crab, Fig. 1). Red king crabs had a higher density than Tanner crabs in the four habitats [Friedman two-way analysis of variance (ANOVA), $p < .05$]. The highest crab density was found in sand-mud habitat for both red king and Tanner crabs. This pattern indicates habitat preference by crabs and has been mentioned in other studies with indirect observations (Bright 1967, Feder and Jewett 1987). Because Tanner crabs have a behavior of burying in the sediment (Stevens et al. 1994) and red king crabs do not, a sand-mud habitat may be more important for Tanner crabs.

A high density of red king crab was also present on debris. Food availability on debris may attract the crabs, because red king crabs are scavengers. Jewett and Feder (1982) reported that sediments and plant materials had a high frequency of occurrence in the stomachs of the red king crab, 34.8 and 33.7%, respectively. Unlike the red king crab, the Tanner crab did not have a high density in the debris habitat. This may be also related to their diet preference. Plant materials had a low frequency of occurrence

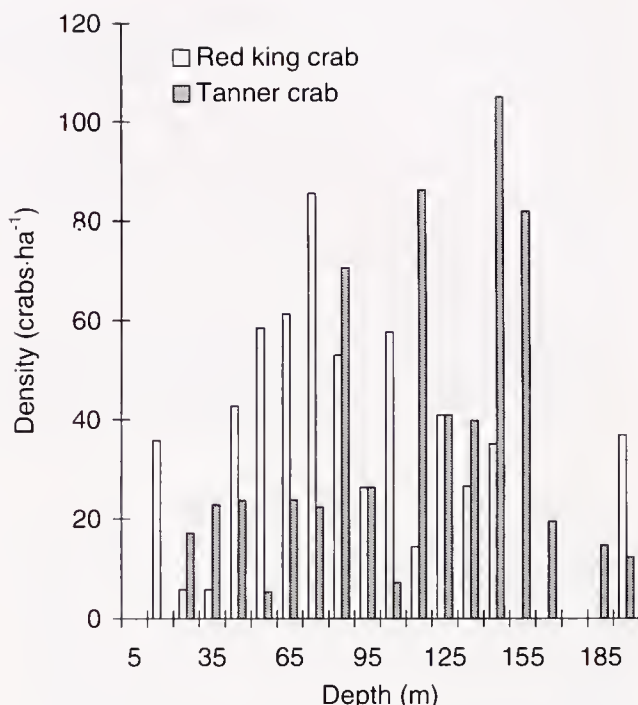


Figure 2. Density distribution of red king crabs and Tanner crabs with depth.

(5.7%), and sediments had a high frequency of occurrence (56.4%) in stomach contents of Tanner crabs (Jewett and Feder 1983).

Crabs Distribution by Depth

Depth was divided into strata of 10-m increments. Red king crabs were observed from 10 to 200 m; Tanner crabs were found from 20 to 200 m. The densities of both crab species increased with increasing depth to a maximum and then decreased at greater depths (Fig. 2). For red king crabs, the maximum density was at approximately 70 to 80 m where 23 red king crabs were observed in 2686 m². Tanner crabs seemed to occur deeper than red king crabs. The highest density of Tanner was at 140 to 150 m where nine crabs were observed in 858 m².

Because red king crabs and Tanner crabs have seasonal migration patterns (Bright 1967, Stone et al. 1992), their depth distribution varies with the season and the locality. Within the same geographic area, Tanner crabs tend to occur deeper than red king crabs (Bright 1967, Otto et al. 1984, Stevens et al. 1996). The dome-shaped depth distribution in our survey implies that both red king crab and Tanner crab have depth preferences, at least during the summer, and red king crabs occupy shallower depths than Tanner crabs within the depth range we investigated.

ACKNOWLEDGMENTS

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HABITAT ESSENTIAL FOR SUSTAINABLE FISHERIES

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ABSTRACT With the passage of the Magnuson–Stevens Fisheries Conservation and Management Act in the fall of 1996, significant new tools exist in the United States to protect and conserve the habitat of marine, estuarine, and anadromous finfish as well as key populations of mollusks and crustaceans. Over the following 24 months, all Fisheries Management Councils are required to amend their fishery management plans (FMPs, covering over 300 species) to identify for each species the essential fish habitat that are those waters and substrate necessary for fish spawning, feeding, and growth to maturity. Threats to habitat and steps necessary to ameliorate those threats must also be identified.

KEY WORDS: Fishery management, habitat, Magnuson–Stevens Act, essential fish habitat

With the passage of the Magnuson–Stevens Fisheries Conservation and Management Act in the fall of 1996, significant new tools exist in the United States to protect and conserve the habitat of marine, estuarine, and anadromous finfish and shellfish. The community of shellfish scientists will be important players both in identifying essential fish habitat and habitat threats, and in monitoring the effectiveness of protective measures once habitat has been identified as essential. This paper presents an overview of the new policies, issues involved in designating habitat as essential, and implications to resource scientists and managers.

Fishery resources of the United States Exclusive Economic Zone are managed by the Secretary of Commerce working through the National Marine Fisheries Service (NMFS) and authorized originally by the Magnuson Fisheries Conservation and Management Act of 1976. The key management tools are fishery management plans that are developed primarily by eight regional fishery management councils. The councils are composed of experts and stakeholders who are nominated by governors of the coastal states in each region and appointed by the Secretary of Commerce. Traditionally, fishery management plans have emphasized allowable catch levels, limitations on fishing effort, and allowable types of fishing gear. Until recently, the Secretary, NMFS, and the Councils have had little authority to influence the quality and quantity of habitat necessary for sustainable fisheries more substantially.

The importance of habitat for the reproduction, growth, and migration of healthy fishery stocks has been acknowledged repeatedly (e.g., Stroud 1992, Sindermann 1996, Langton et al. 1996, NMFS 1996). The congress of the United States also recognized the importance of habitat with the passage in the fall of 1996 of the Magnuson–Stevens Fisheries Conservation and Management Act (16 U.S.C. 1801 *et seq.*) and provided important new approaches for managing the habitat of marine, estuarine, and anadromous finfish, mollusks, and crustaceans.

Habitat is generally thought of as the place where an animal is found (e.g., estuaries, deltas, channels, grass beds, convergence and turbidity zones, fronts separating water masses). Fish and shellfish use habitat for spawning, feeding, nursery, migration, and residence functions; most habitats perform only a subset of all of these functions (Edwards et al. 1992). The habitat of a fish or shellfish may change with changes in its life history stage, seasonal and geographic distributions, abundance, and interactions among species. Indeed, as scientists sample new areas and gain a better understanding of the ecology of an aquatic animal, the perception of its habitat may change (Peters and Cross 1992). Not only are the

location and type of habitat important, but also its attributes and functions. If habitat functions are reduced or lost, then the needs of fish and shellfish cannot be met, and the ability of the habitat to support those living resources is reduced (Edwards et al. 1992).

By October 1998, all Fisheries Management Councils are required to amend their fishery management plans (covering more than 600 federally managed fishery stocks; Fig. 1) to identify and describe for each species their essential fish habitats (EFH), which was defined by the U.S. Congress to be “those waters and substrate necessary for fish for spawning, feeding, or growth to maturity.”

The Magnuson–Stevens Act requires that EFH be identified and described for each of the federally managed species. Essential fish habitat is identified by its geographic limits, which will vary depending upon the amount and kind of data available. The identification includes geographic maps at various time and space scales for each of the life history stages. Essential fish habitat is described by specifying those components of the ecosystem that must be present for different life history stages of a species to occur. In practice, essential habitat can be described by matrices of environmental variables by life history stage (Cross et al. 1998).

The designation of EFH will involve the identification and description of habitat requirements and the characterization and mapping of habitat for the critical life stages of each species. In addition, threats (including damage from fishing gear) to essential habitat will be identified, and conservation and enhancement measures will be proposed. After the amendments are in place, consultations may be required between the councils and federal agencies whose actions may have an adverse impact on essential fish habitat to mitigate the impacts.

The Magnuson–Stevens Act includes substantial new provisions to ensure the conservation and management of EFH once these areas are designated in fishery management plans approved by the Secretary of Commerce. Federal agencies that authorize, fund, or undertake actions that may adversely affect EFH must consult with NMFS to evaluate the effects of their actions on EFH and the associated life stages of finfish, mollusks, and crustaceans. These provisions ensure that actions that require a federal permit or license or that are funded or implemented by a federal agency must account for potential harm to federally managed species of fish and their essential habitats. In turn, NMFS is required to provide conservation recommendations to federal and state agencies to assist the agencies in avoiding, minimizing, or offsetting any adverse effects to EFH (Cross et al. 1998).

Information from shellfish scientists throughout the country



Figure 1. Fishery management plans.

will be needed to identify essential fish habitat and habitat threats accurately, and the cooperation of shellfish managers at all levels will be needed to monitor the effectiveness of protective measures that come into force once habitat has been identified as essential. The designation of EFH for fish and shellfish species will involve the identification and mapping of habitat requirements for all critical life stages of each species. In addition, threats to all essential

habitat types will be identified, and conservation and enhancement measures will be proposed. Given the important function that shellfish habitat plays in the key life stages of many species, such habitats are prime candidates as essential fish habitat. The new policies to implement the Magnuson-Stevens Act will provide important tools to federal and state natural resources managers for the conservation of shellfish habitat.

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LASER LINE SCAN SURVEY OF CRAB HABITATS IN ALASKAN WATERS

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ABSTRACT In 1996, the Alaska Fisheries Science Center/Alaska Department of Fish and Game, conducted a study to demonstrate the feasibility of using a laser line scan (LLS) system to detect physical and biological changes in bottom habitat attributable to trawling and to collect data for stock assessment. Surveys were conducted near Kodiak Island and in the Gulf of Alaska. Objectives were to: (1) compare LLS imagery with video and side scan over known bottom; (2) determine if new and old trawl tracks were detectable; (3) assess stocks of Tanner and red king crabs. The LLS excelled at imaging requirements and in identifying bottom fauna.

KEY WORDS: Laser, laser line scan, crab habitat, Alaska

INTRODUCTION

A laser line scan (LLS) survey of crab habitats in Alaskan waters was conducted in conjunction with the NMFS Alaska Fisheries Science Center to provide an over-all assessment of effects of fishing practices on crab habitat. Specific objectives of the investigation were to: (1) compare LLS with video and side-scan sonar imagery; (2) determine if LLS can distinguish old from new trawl marks; and (3) to determine if LLS can distinguish and enumerate crab species.

The LLS survey was conducted in combination with trawling in areas known to contain crabs including Cheniak, Woman's, Ugak, and Marmot Bays and nearby offshore areas. This initial report focuses on the ability of the LLS to locate and image crabs; the comparative analyses stated in the objectives above will be the subject of a later report.

MATERIALS AND METHODS

Information regarding the survey parameters, operational characteristics, system design and data-processing steps are briefly explained below. Greater detail on the operational principles of the LLS system, method of deployment, data acquisition, and post-cruise data analysis protocols may be found in Rhoads et al. (1997).

Survey Parameters

The survey was conducted in the general vicinity of Kodiak Island. The 70-ft survey vessel *Resolution*, provided by State of Alaska, met the minimum size requirement deemed necessary to conduct the investigation considering expected sea state conditions and hardware requirements for the survey. Eight days of surveying were conducted. For LLS investigations, the towing altitude of the scanning device was 3 m above bottom (mab) in the bays and up to 8 mab in adjacent waters. The survey depth was adjusted to optimize for desired imaging resolution and areal coverage rate as discussed below.

Operational Characteristics

The laser line scan tow body system was designed to provide seafloor images with high coverage rates (working tow speed 1 to

6 knots). The swath of the imaged area along a transect is approximately 1.4 times the altitude above seafloor, affording image resolution in cm to mm range (Table 1). By comparison with SVHS video format, comparable image quality is obtained at a viewing range which is 5 times conventional video. Survey depths up to 2,000 m are possible when deployed via ROV; towed configurations are cable-limited to 600 m.

System Configuration

The LLS system is composed of an underwater optical sensor consisting of solid-state Nd-YAG (blue-green) laser with two 4-faceted rotating mirrors and a synchronized receiver (Fig. 1). The topside control console provides control of LLS power requirements, data management and display, scan rate, and aperture position. The LLS data are recorded in digital form on hard disk or as live video and stored on cassette. A hydrodynamic tow body including umbilical cable and power supply completes the hardware configuration.

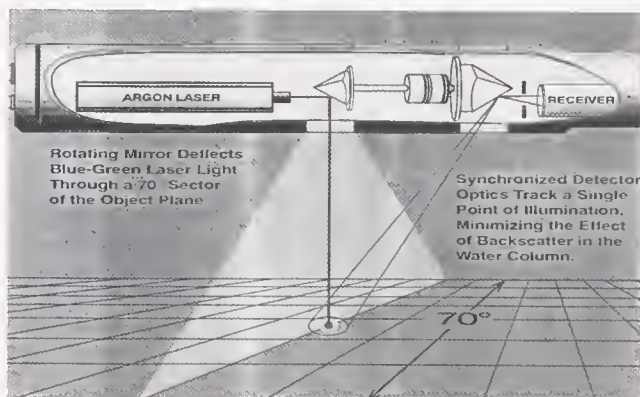
LLS System Data Processing and Display

Image data collected from the LLS system is digitized using RasterOps video capture hardware. Video images of each survey

TABLE 1.
Representative system performance of the laser line scan system.

Water Clarity	Typical Imaging Range	Swath Width	Areal Coverage Rate (3 kts)	Sampling Resolution (2,048 pixels)
Very clear (Hawaii)	45 m	65 m	346,000 m ² h ⁻¹	3 cm
Clear (Eolian Island)	22 m	30 m	161,000 m ² h ⁻¹	1.5 cm
Moderate (Massachusetts Bay)	9 m	13 m	69,000 m ² h ⁻¹	0.6 cm
Poor (Boston Harbor)	3 m	4 m	23,000 m ² h ⁻¹	0.2 cm

SM2000 Underwater Laser Line Scan System Specifications:	
Operating Depth	5,000 feet
Sensor Altitude	8 to 150 feet (dependent on water quality)
Survey Swath Width	10-210 feet (dependent on water quality & survey requirements)
Survey Speed	0.5 to 6 knots
Angle Range of Scanner	15° to 70°
Resolution	0.5 inches at 100 ft
Laser Wavelengths	488nm & 514.5nm (Argon Ion Gas Laser)
Optical Power (in water)	1.5W
Data Output	RS170/CCIR Mono Standard Video
Data Display	Video Monitor
Power Required (wet end)	120V, 3ø, 5kW
Size (wet end)	69 inches long x 11 inches diameter
Weight (wet end)	170 lbs. (in water); 390 lbs. (in air)



The SM2000 is a synchronous scanning system. An Argon ion laser continuously scans a narrow beam across a selected area with a 70° field of view, illuminating only a small spot at a time. A high sensitivity receiver tracks the beam with a narrow view angle, counteracting the effect of backscatter in the water column. The receiver's output is digitized and stored in a digital image buffer. As the sensor moves forward, new lines of data are stored in memory and the resulting imagery is real-time standard video which can be recorded on conventional video equipment.

Figure 1. System components and specifications of the SM2000 laser line scan receiver.

line are stored in series of standard image files, with real-time correction of the image for heading, speed, and slant range. As a result, the video image can be considered as a continuous mosaic of pixels in rectified spatial geometry. Pixel size can be calculated in postprocessing by taking into account towfish altitude to estimate swath width, which when divided by the number of pixels in line, yields pixel dimensions. ARCVIEW software is used to transfer the image data and associated marker files into Geographical Information System (GIS) format for distribution on CD-ROM medium. The final product allows the user to point-and-click on geographic locations for laser image(s).

RESULTS AND DISCUSSION

Results of the investigation revealed the extended capability of the LLS system to locate king crab populations as well as to characterize habitat types to which they were (and were not) associated. An example image of crab aggregation behavior is shown

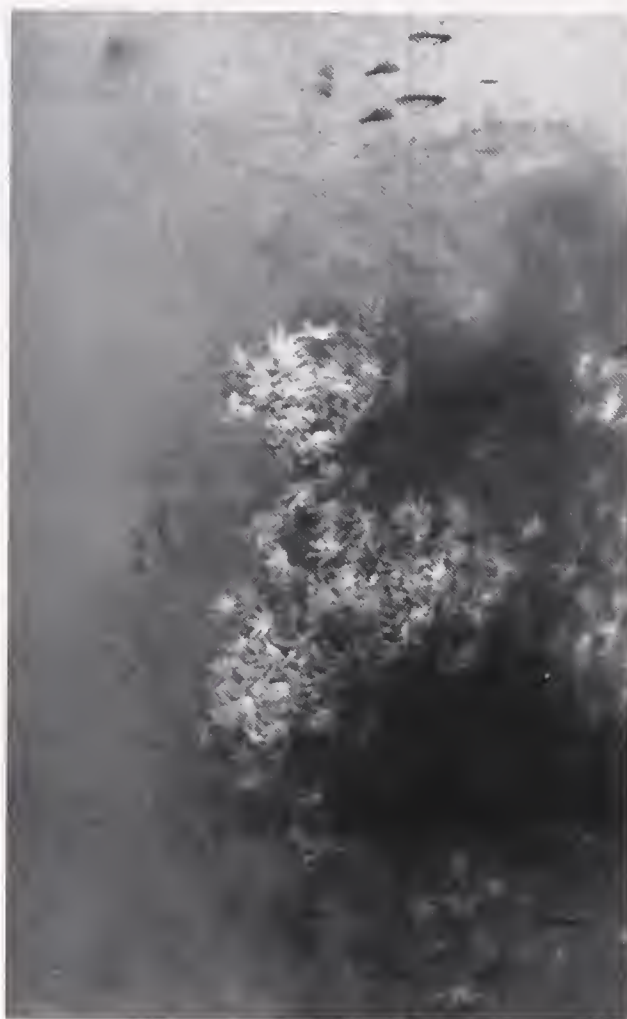


Figure 2. Example image of crab aggregation behavior in Alaskan waters. Approximate image width is 4 m.

in Figure 2, where it is evident that hundreds of individuals are clustered together in a soft sediment burrow. The apparent banding in the image is an artifact of the individual line scans used to construct the composite image. Although this seemingly simple task could also have been accomplished using traditional video or diver survey, these alternative approaches would have required a far greater fiscal investment and a greatly expanded schedule.

Figure 3 depicts a section of the video image showing a kelp bed in shallow water in the vicinity of Kodiak Island. There also appears to be one or more flat fish in the image that are "buried" in the soft sediment. Upon closer inspection, several anenomes/starfish can be seen intermingled with the kelp. Figure 4 also depicts crab clusters but at a higher altitude above bottom (8 m). Finally, Figure 5 shows an image of a solitary fish in midstroke, along with the shadow cast by the laser. The ability to "sample" fish distribution with minimal intrusion should prove to be a valuable attribute of the instrument that cannot be matched by other light-based imaging methods.

Historically, rapid mapping of the seafloor for environmental assessment has been exclusively done by towed acoustic side scan (Rhoads et al. 1994). Imaging applications have included



Figure 3. Kelp beds in shallow waters of Kodiak Island. Approximate image width is 4 m.

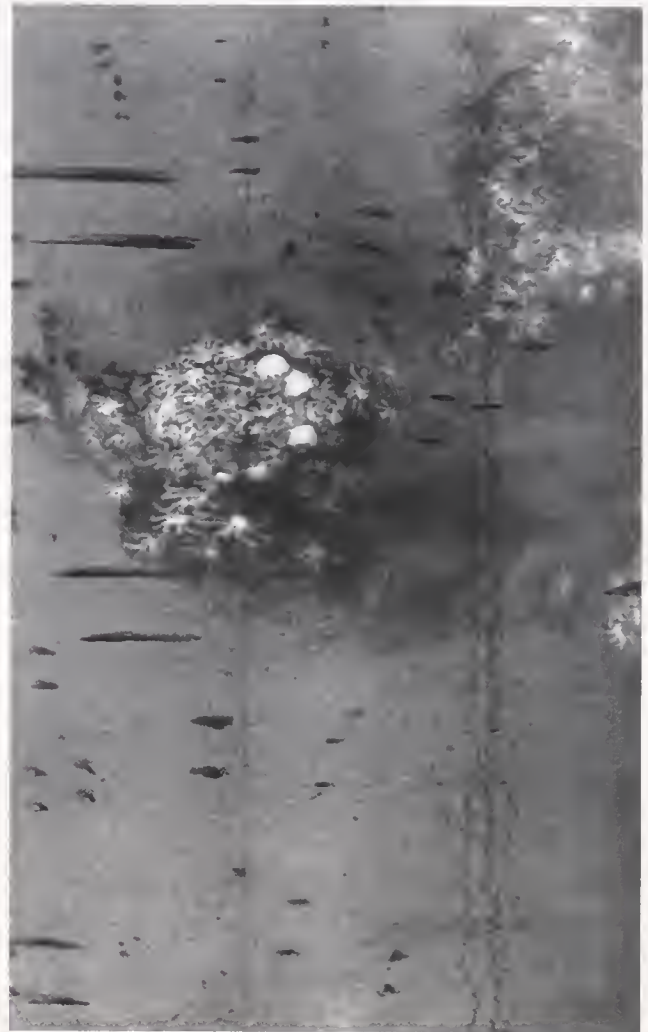


Figure 4. Two crab clusters observed off of Kodiak Island. Approximate swath width is 10 m.

environmental survey and mapping (i.e., seagrass, habitat and fish stock assessment, archeological surveys, dredged material monitoring), cable/pipeline survey inspections, search-and-locate missions [e.g., vessel and aircraft accident investigations, hazardous/industrial waste container surveys, unexploded ordnance (UXO) detection and intelligence gathering]. Swath width of a conventional 50 kHz acoustic side scan is ~10–15 times towfish altitude; whereas, LLS system coverage at 1.4 times is an order of magnitude less. However, object resolution of a side scan is on the order of ~100 cm; whereas, the LLS system has the potential for object resolution of a few millimeters to centimeters (Hellem et al. 1994). Hence, the choice of imaging method is typically a balance between the need for areal coverage and certainty of target location/identification.

In the initial phases of the survey, a rapid screening exercise is often conducted wherein a high-altitude "first pass" with an acoustic sensor is performed to narrow the over-all study area as well as to evaluate the risk of flying the LLS system nearer the bottom. This is particularly important in areas that are uncharted or in areas where there are wrecks, debris, snags, or high topographic relief. In addition, the altitude of the towfish is dependent on water clarity and must be less than about five opti-

cal attenuation lengths. In New England and New York waters, typical tow fish altitudes are between 8 m at Cohasset Ledge, Massachusetts (rock debris with little suspended material above the bottom) to about 2.5 m in the muddy basin of central Long Island Sound and within the Hudson Canyon (Inglin 1994). The logistics of this survey approach are minimized, because the towfish can incorporate both the LLS and conventional acoustic side-scan/bathymetry sensors.

Picture quality of video images in the present investigation confirms the utility of the system to map gradients in bottom type that may affect the distribution of such biological resources as crab, demersal fish, and other megafauna and macrofauna. Quantitative data can be acquired by a frame-by-frame analysis for existence of particular target types (e.g., crab clusters), and graphically displayed as transect scatter plots, stick diagrams, histograms, or frame-averaged transect trends. Correlation and association techniques as well as multivariate pattern recognition (community analysis) may also be utilized to investigate the relationship between the distribution of fisheries resources and physical parameters of the benthic environment, such as sedi-



Figure 5. Solitary fish with shadow cast by the laser light of the LLS system instrument.

ment type, dredged material distributions, and seafloor topography.

The latest version of the LLS system, the SM2000, is manufactured by Westinghouse and owned by SAIC. SAIC has also developed a neutrally buoyant vehicle to house the laser, which can attain depths in excess of 1,500 m and maneuver up, down, left, and right. This extreme depth and flight capability makes this system one of the only high-resolution imaging technologies presently available that can be used for detailed, high areal coverage of deep-water habitats. As with the shallow water system, mosaics assembled from overlapping imaged transects or individual frames can be generated for display and presentation purposes.

The LLS system-based images of the seafloor have been proved to be very effective in public relations presentations to demonstrate the effectiveness of using a coarse-grained material to cap fine-grained contaminated material at dredged material disposal sites (Inglin 1994). In this instance, the high-quality images spatially orient the viewer relative to the ambient bottom, disposal mound flanks, and mound apex in a format similar to conventional communications media and makes meaningful what was before a technical term, line drawing, graph, or table.

Clearly, the rapid survey rate at high image quality is a major strength of the LLS system. On a day-rate cost basis, the LLS system is comparable to other high-resolution mapping techniques, but when the objective is obtaining continuous, photographic-quality images over large areas, the cost of using the LLS system per unit area is 10 to 40 times less than ROV or camera sled technologies (Rhoads et al. 1997).

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MONITORING BENTHIC HABITATS IN A MARINE NATURE RESERVE

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ABSTRACT Acoustic and underwater photographic techniques have been used to assess the impact of commercial trawling on the benthic habitats of a Marine Nature Reserve. The results have been used as part of the management of the area. The further application of these techniques as tools for fisheries research and environmental monitoring is discussed.

KEY WORDS: Marine Nature Reserve, *RoxAnn*, side-scan sonar, fisheries impact, Strangford Lough

INTRODUCTION

Strangford Lough is a narrow arm of the sea with a maximum depth of 60 m, on the east coast of Northern Ireland (Fig. 1). Strangford Lough is regarded as one of the most environmentally important areas in Northern Ireland, being internationally noted for its biodiversity. Much of the foreshore is designated under current legislation as an Area of Special Scientific Interest (ASSI) and includes several nature reserves. The Department of the Environment for Northern Ireland has also designated the Lough as a Marine Nature Reserve.

Modiolus modiolus in the Lough do not have a uniform density but occur in discreet clumps of 5 to 30 individuals, each clump being separated by distinct areas of mud and silt (Roberts 1975). The resulting clumps provide a biotic hard substrate in an area where such a substrate would not normally exist and so provide a habitat for a much wider range of species (Erwin 1977). These communities are currently under review for designation as Special Areas of Conservation under the EU Habitats Directive (92/43/EEC), which require that appropriate management plans be prepared.

In recent years, conflict has arisen between conservation groups and commercial fishing interests over perceived damage caused to the *Modiolus modiolus* communities. The fishery is based around the queen scallop *Aequipecten opercularis*. Initial studies (Service 1990) using a remotely operated vehicle (ROV) indicated that a degree of impact from the fishery was occurring and a 5-point classification of the Lough habitats was created (Table 1).

This paper describes how remote sensing in the form of direct video imaging and acoustic mapping has been used as a management tool for the assessment of the status of benthic habitats in a marine nature reserve. In Strangford Lough three main techniques have been applied: (1) direct video and photographic imaging using a towed camera sledge; (2) side-scan sonar; and (3) the acoustic ground discrimination system, *RoxAnn* (Marine Microsystems, Aberdeen, Scotland). These methods, coupled with the use of Geographical Information System (GIS), have enabled the extent of the benthic communities of interest to be mapped and the degree to which trawling activity has made an impact on them to be assessed.

METHODS

The techniques used in this study have been previously described elsewhere: *RoxAnn*, Magorrian et al. (1995), side-scan sonar and ROV, Service and Magorrian (in press) and underwater video analysis, Magorrian and Service (in press).

RESULTS AND DISCUSSION

Side-scan sonar surveys carried out during 1990 and 1993 readily identified the presence of paired scars on the seabed that

could be attributed to the action of trawl doors. The information from the two surveys was then plotted onto a GIS. The *RoxAnn* survey clearly identified the distribution of the major communities associated with *M. modiolus*. By combining the results from the *RoxAnn* survey with the data from the side-scan sonar surveys it proved possible to quantify the degree to which the *M. modiolus* communities were being affected by trawling (Fig. 2, Table 2). This information has subsequently been used to zone the trawl fishery in the lough.

Further analysis of the underwater video and stills data aimed at quantifying the impact of trawling at a species and community level were then undertaken. Details of these techniques can be found in Magorrian and Service (1998). Multivariate analysis of the video data produced a pattern of community analysis that was in general agreement with subjective scoring system utilized in Table 1.

OTHER STUDIES

Outside the area of habitat management, *RoxAnn* is now being used as a fisheries management tool to define the extent of certain commercial shellfish beds. It has proved possible to map the extent of mussel *Mytilus edulis* beds in Lough Foyle and Carlingford Lough (Fig. 1) so that aquaculture licenses may be managed appropriately.

In the wider context, the commercial fishery for scampi or Norway Lobster *Nephrops norvegicus* is the most important to the Northern Ireland fleet. However, stock assessment of this species is difficult because of the life style of the organism. This animal

TABLE 1.

Habitat scores for *M. modiolus* communities.

Habitat Category	Description
1	Area does not form part of the <i>M. modiolus</i> communities. Mainly soft muddy bottom with burrows typical of <i>Nephrops norvegicus</i> communities
2	Soft bottom with dead broken shell, partially buried in the soft sediment. Area often is colonized by tunicates and associated epifauna.
3	<i>M. modiolus</i> beds affected by trawling in the past but largely intact. Emergent epifauna clearly visible.
4	<i>M. modiolus</i> beds moderately affected by trawling with shell debris evident. <i>M. modiolus</i> not forming discrete clumps, more scattered, flatter appearance. Some epifauna still persisting but emergent epifauna virtually absent.
5	<i>M. modiolus</i> beds are heavily impacted by trawling. Few intact <i>M. modiolus</i> , lots of shell debris. Epifauna is sparse.

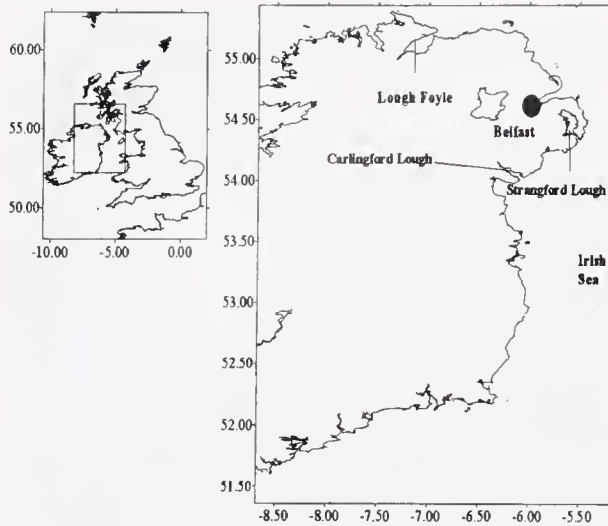


Figure 1. (a) Strangford Lough, situated on the east coast of Northern Ireland.

TABLE 2.

A comparison between the area exhibiting trawl impact from the 1990 and 1993 side-scan sonar surveys.

	1990 (km ²)	1993 (km ²)
Area surveyed	10.6	13.3
Area showing evidence of trawling	3.3	4.2
Area within <i>M. modiolus</i> communities showing evidence of trawling	2.8	2.7
Area within <i>M. modiolus</i> / <i>C. varia</i> communities showing evidence of trawling	2.7	2.6

burrows in muddy sediments emerging only to feed, normally at dawn and dusk. Egg-bearing females do not leave their burrows. One technique to obtain fishery-independent population estimates is by counting burrow density using underwater video or still photography. Recent studies have developed the hierarchical keys that can be used to identify the organisms responsible for producing different burrows (Marrs 1996).

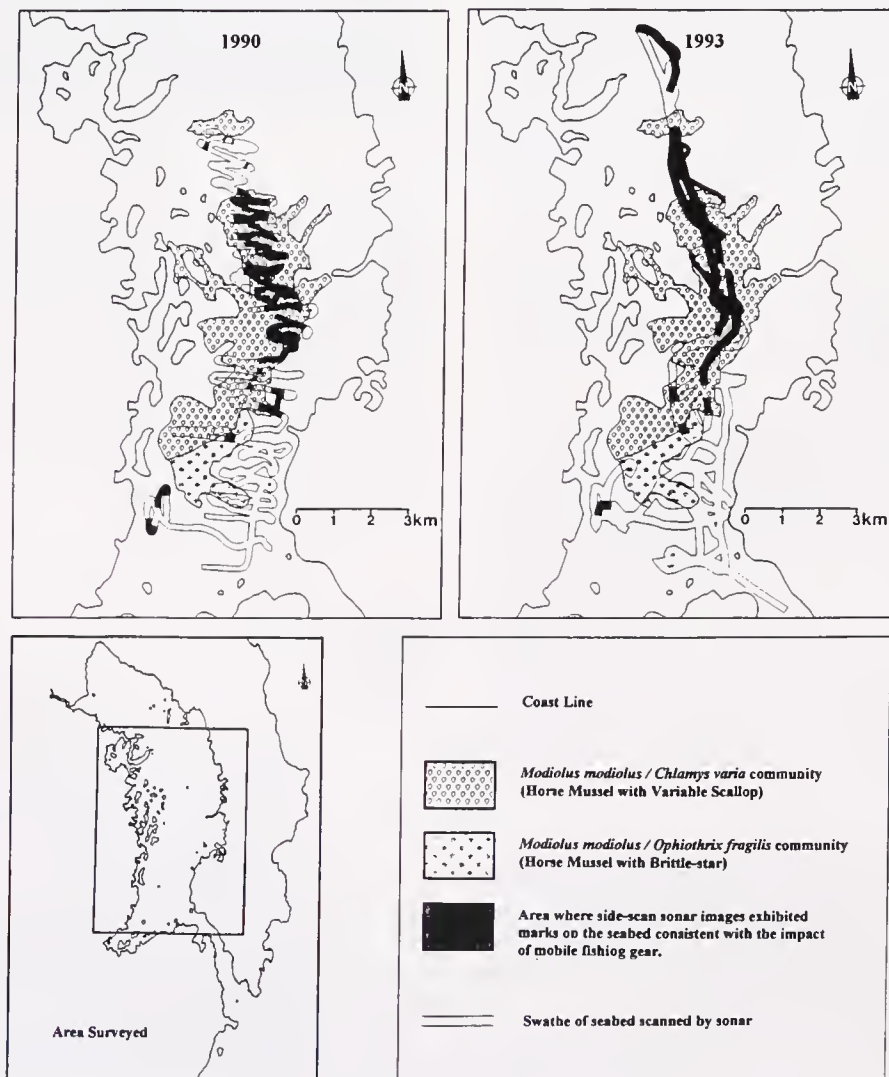


Figure 2. (a) The 1990 side-scan sonar survey of Strangford Lough; (b) 1993 side-scan sonar survey of Strangford Lough.

In the wider context, it has been recognized that epifauna are generally less well studied components of the benthos in terms of community structure and its response to anthropogenic impacts. It has been recommended that appropriate methodology be developed for the quantitative assessment of epifaunal communities

(Anon 1993). As discussed above, Magorrian and Service (1998) have applied a semiquantitative technique, the visual fast count system (Kimmel 1985), to video images collected using a camera sled to assess the impact of trawling on epibenthic communities.

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UTILIZATION OF GIS AND GPS FOR SHELLFISH GROWOUT SITE SELECTION

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ABSTRACT The optimization of survival and growth of soft-shell clams in eastern Maine depends upon numerous physical characteristics, including bathymetry, bottom type, intertidal location, water currents, temperature, and planktonic concentrations. This information can be organized into a Geographical Information System (GIS). The analysis of this information along with a biological field experiment is used to predict and map growth rates for potential seeding sites for juvenile shellfish throughout Mason Bay, Maine. Elevation measurements were organized into a triangular irregular network (TIN) data model to estimate continuous elevations. Current vectors were estimated via a numerical model. Aerial infrared photographs, chart information, and bottom type were additionally integrated.

KEY WORDS: soft-shell clams, Geographical Information System, bathymetry, water currents

Commercial harvests of soft-shell clams have declined by over 400,000 bushels since the 1970s in Eastern Maine. Traditionally, Washington County has accounted for nearly 60% of state landings, but presently is now only responsible for 17% of Maine's harvests. In some communities, hatchery-reared juveniles have been seeded on mudflats to replenish clam populations. The optimization of growth and survival of these juveniles depends upon numerous physical characteristics including: bathymetry, bottom type, intertidal location, water currents, temperature, and planktonic concentrations. Infrared aerial photographs along with maps of topography, bathymetry, and sediment type were incorporated into the *MapInfo* GIS (Geographical Information System) to describe these physical parameters of an intertidal bay in Washington County. However, data needed to determine intertidal elevations and current rates are lacking.

To obtain this information, elevations from NOAA nautical charts and GPS (Global Positioning System) measurements were included as data layers in *MapInfo*. Initial GPS measurements were taken with Trimble GeoExplorer 6-channel parallel/sequential hand-held receivers for a duration of 45 minutes. Using carrier phase GPS with a local base station (0.65 km baseline), we obtained accuracies comparable to subtidal elevations from NOAA nautical charts. The error associated with a single measurement was 0.37 m. This accuracy was found through either one 40-min carrier phase GPS measurement or by averaging four 10-min measurements. Additional GPS elevation measurements were taken following the low waterline on the south side of the bay. Using an aerial photograph displaying the low waterline, the location of the low waterline on the northern side of the bay was estimated.

With elevation points throughout the bay from NOAA charts, GPS measurements, and the low waterline, a grid of elevations for

the entire bay was constructed. Each elevation point became a node in a set of interconnected triangles representing the surface of the entire bay. The vertices of the triangles became the geographic locations, or the *x* and *y* coordinates, and the elevations, or *z* coordinates, became data for the node. With this information, elevations within each triangular plane could be determined. This series of triangles is known as a triangular irregular network or TIN (Fig. 1).

A C++ program was coded, using the list of nodes and triangles as input to generate a grid of elevations/depths throughout the bay. The grid consisted of 7,600 square cells, with 36.125 m sides and elevation estimates for the midpoints. Elevations relative to mean sea level are displayed in false color in Figure 2 created by *Vertical Mapper* add-on software for *MapInfo*.

The Princeton Oceanographic Model (POM), a numerical computer model, used the elevation grid and tidal range to estimate the direction and magnitude of current velocities throughout the bay. Instability was found within the model because of the continual flooding and drying of the intertidal grid cells. The model was modified by incorporating a flag cell to indicate the status of each cell. The *u* and *v* components of velocity and free surface elevation were also calculated. As a cell became dry on the ebb tide, the cell was excluded from the model's calculations. On the flood tide, a cell's status would become wet as the water level exceeded a specified level. These cells were also excluded. Calculations were executed for every 1-second of the tidal cycle and output every 10 minutes.

Output data from the POM were translated by a series of C++ programs for the *MapInfo* GIS. *Vertical Mapper* was used to create a false color image (Fig. 3) of average current velocities for a 4-m tide throughout the bay. As the tide flows westwardly into the bay, tidal currents diminish. This is also shown in Figure 4. *Vertical*

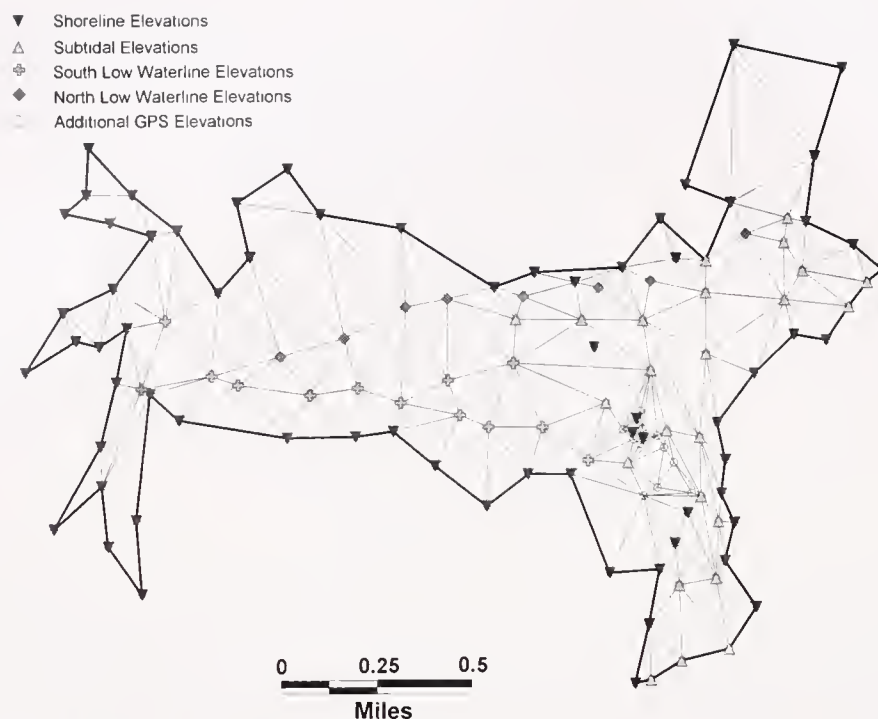


Figure 1. The TIN output of Mason Bay, Maine: a triangular irregular network (TIN) was created by connecting points of estimated elevations throughout the bay. The small triangles represent locations from NOAA nautical chart #13325. Carrier-phase GPS measurements were taken on the southern side of the bay following the low waterline (crosses), and the elevations of the northern low waterline were estimated using an aerial photograph (diamonds). The circles represent GPS measurements from a biological experiment.

Mapper was used to create this map of symbols representing the magnitude and direction of flow at a particular time in a tide cycle.

Cross sections also support the representation of diminishing flow in the westward region of the bay (Fig. 5). These images were stored as layers in the *MapInfo* GIS and can be viewed with any of the other layers from the bay.

Because the flow of water can affect the vertical and horizontal distribution of phytoplankton in the water column, understanding

the magnitude and direction of currents is of extreme importance when selecting a site for aquaculture. A diminishing flow may limit the amount of food available to infaunal filter feeders; whereas, an extremely strong current may cause a retraction of the siphons. Both conditions would inhibit growth by limiting the amount of food ingested by the animal. Thus, an additional layer of maximum current velocities for the bay was created in the GIS. At specific times during a tidal cycle, a counterclock-

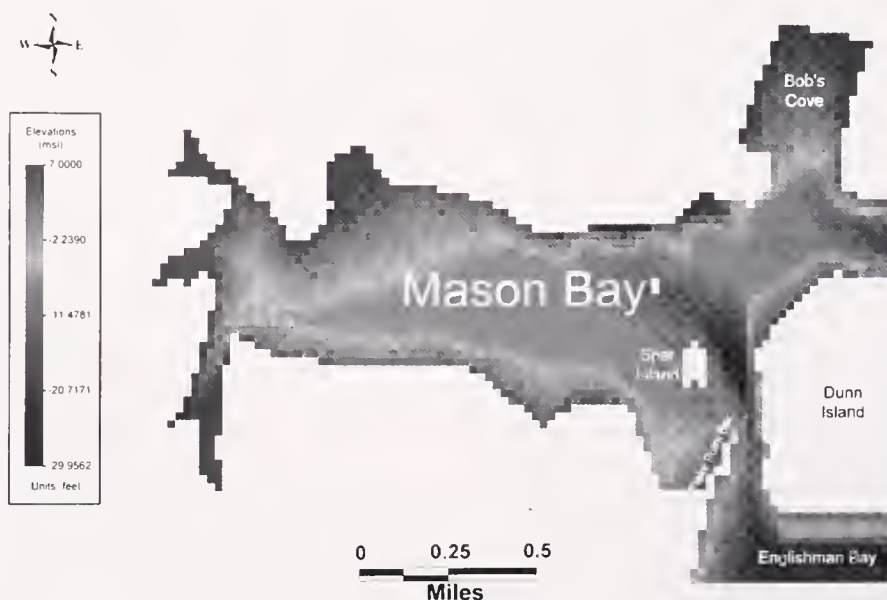


Figure 2. Elevations in Mason Bay; the elevations estimated from the TIN were used in *Vertical Mapper* to create a false color image.



Figure 3. Average current velocities in Mason Bay; the grid from the Princeton Oceanographic Model representing current velocities for a 4-m tide was used to create a false color image in *Vertical Mapper*. Velocities diminish as the water flows westwardly.

wise eddy develops on the eastern side of Spar Island (Fig. 6). In this figure, the direction of the arrowhead represents the direction in which the current is flowing, and the size of the arrow is proportional to the magnitude of the current. The effects of this eddy on growth and survival are uncertain and remain to be studied.

Using this physical information of the bay along with knowledge of biological factors affecting shellfish growth and survival, locations meeting specific criteria may be determined through a query in *MapInfo*. Criteria needed to query data for potential sites for soft-shell clam growout would include data on bottom types, intertidal elevations, and average and maximum current velocities.

A sample query for *Mya arenaria* would include the following specifications: (1) a bottom type of mudflat; (2) an elevation between -4 and -6 ft msl; and (3) an average current velocity between 0.09 and 0.10 m/sec.

The sites or cells meeting these criteria can be displayed in either table or map format. A map identifying the selected sites meeting the above criteria are displayed in Figure 7. The query may be layered on top of an aerial photograph to determine the accessibility to the sites by road.

Use of GIS and GPS in intertidal regions has great potential for habitat description and management in the shellfish aquaculture industry. Intertidal elevation and current data are presently

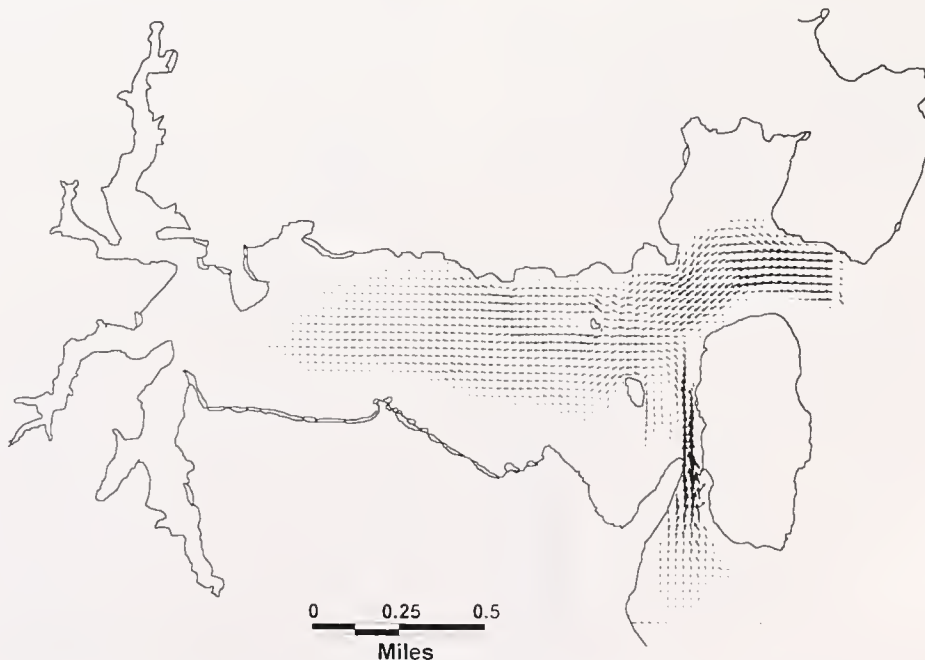


Figure 4. Mason Bay on the flood tide; the arrows represent the direction and magnitude of the flow three hours after low water. The lengths of the arrows are proportional to water displacement per minute. The arrows become smaller as the water flows to the west.

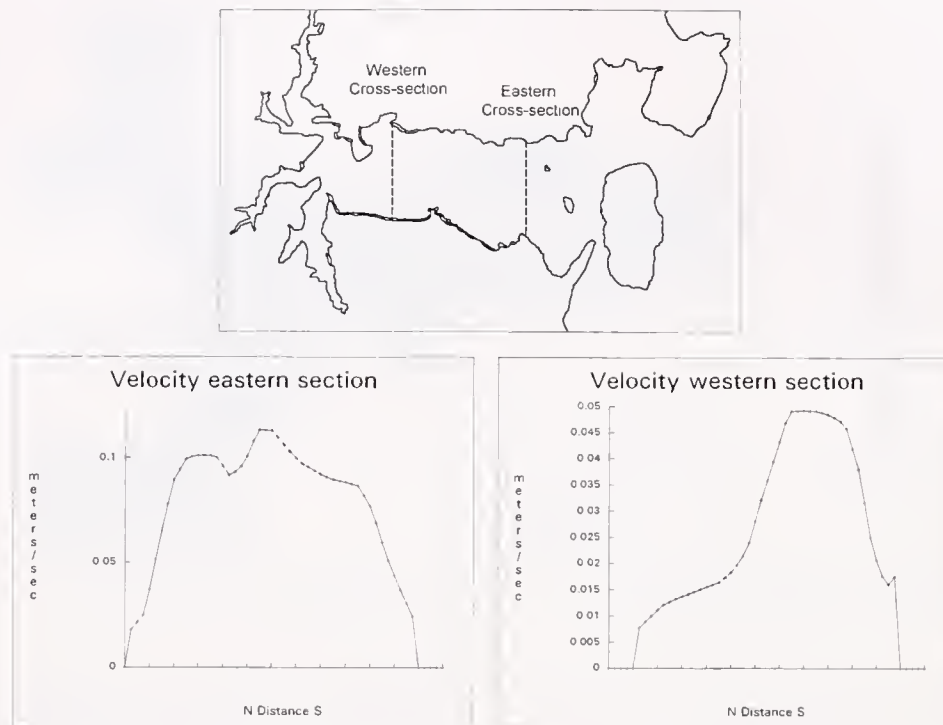


Figure 5. Cross sections of velocity averages; velocity in the eastern section is twice as fast as in the western section. A slight reduction in velocity occurs in the eastern section due to the presence of a small island.

limited, but can be determined through GPS, numerical computer models, and a Geographical Information System. Phase processing of the carrier signal is required to measure intertidal elevations with accuracy comparable to NOAA nautical charts. A

TIN, using datapoints within the bay, can be used to generate a grid of elevations for the entire bay. This grid can then be used by a numerical computer model, such as the POM, to obtain the direction and magnitude of currents at the midpoint of each

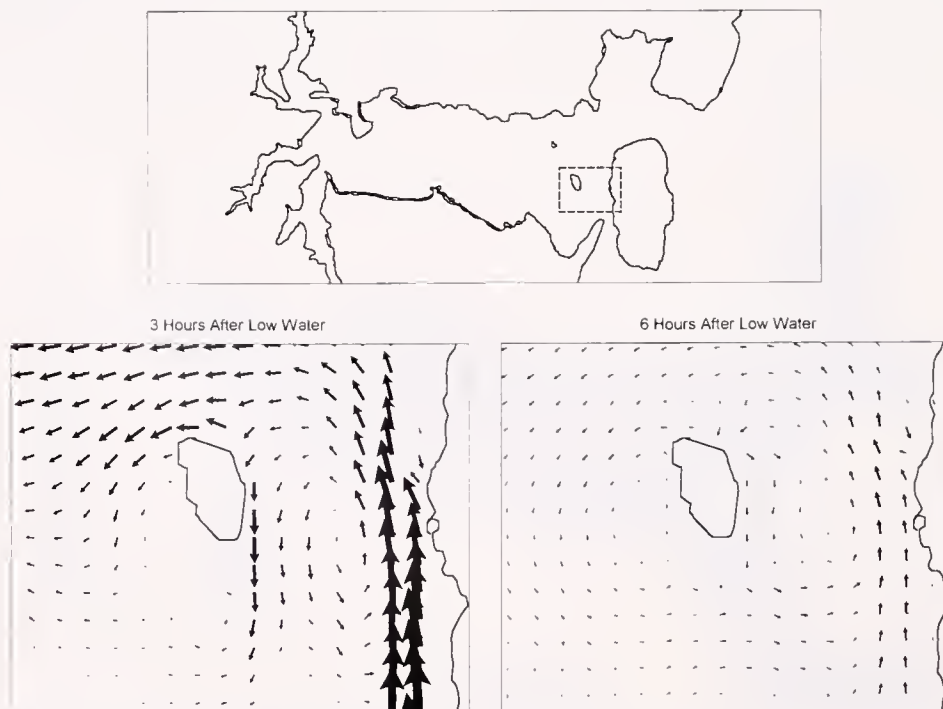


Figure 6. Eddies in Mason Bay; a large eddy, with varying magnitudes, is present in the bay at various times during a tidal cycle. The eddy is located between Spar and Dunn Islands.



Figure 7. Query results; the output from a *MapInfo* query is displayed with stars. Each star is a location satisfying the requirements for elevation, average current velocities, and bottom type.

grid cell. Elevations and currents can be easily imported into a GIS to use in the spatial description of the bay. Additional layers, such as temperatures, salinities, population densities, and phytoplankton concentrations could be created to enhance the physical description of the bay. A final query on all the data is needed to locate sites for the growout of bivalve seed. Properly utilizing GIS

and GPS should be effective tools for managing clam populations in Maine.

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SOUND PRODUCTION BY WHITE SHRIMP (*PENAEUS SETIFERUS*), ANALYSIS OF ANOTHER CRUSTACEAN-LIKE SOUND FROM THE GULF OF MEXICO, AND APPLICATIONS FOR PASSIVE SONAR IN THE SHRIMPING INDUSTRY

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ABSTRACT Research concluded that nonstridulating pink shrimp (*Penaeus duorarum*) in the Gulf of Mexico did not make sound in the sonic range, but might produce ultrasonic sound via friction between parts of the exoskeleton. Recordings of “frying” noises over known penaeid shrimp beds led to speculation that shrimp were mechanically producing this portion of the ambient noise, and passive sonar might be used for shrimp detection and population estimations. Results showed that captive shrimp produced broadband impulsive signals (2 to 8 kHz) primarily in association with eating. Spectral comparison suggest that field transients and captive shrimp sounds are similar.

KEY WORDS: Shrimp, penaeids, passive sonar

It is apparent that catch-per-unit-effort (CPUE) has declined over the past three decades in the shrimp fishery of the Gulf of Mexico (Onal et al. 1991, Gulf of Mexico Fishery Management Council 1996). It is unclear what is occurring on the shrimping grounds, but both shrimp fisherman and agencies that monitor the shrimp fishery would benefit from the development of a tool for the remote sensing of shrimp populations and real-time population assessment. No remote detection methods for locating shrimp populations exist at present, resulting in the continued use of random trawling with its associated high incidence of unnecessary by-catch and habitat destruction. A variety of calculation methods exist for density assessment, but they also rely on random trawling to collect the catch data needed for computation. Furthermore, these methods are not in real-time, and their validity rests on the assumption that equilibrium exists in shrimp populations. One tool that holds promise as a solution for these problems is passive sonar.

Past research concluded that pink shrimp (*Penaeus duorarum*) of the Gulf of Mexico shrimp fishery did not make sound in the sonic range, but might produce ultrasonic signals via friction between parts of the exoskeleton (Gehring 1971). This form of sound production, stridulation, may produce either sonic or ultrasonic sound, but it is not known to exist in any of the gulf shrimp fisheries species (Dumortier 1963). However, in 1992, members of the Minerals Management Service “GulfCet” project (Davis and Fargion 1996) frequently encountered “frying” noises while recording cetacean sounds in the northwestern Gulf of Mexico (Evans, pers. comm.). Given that the location and distribution of the frying noises corresponded to known royal red shrimp (*Pleoticus robustus*) habitat, Evans (pers. comm.) hypothesized that large populations of that species, morphologically similar to penaeids, were responsible for the din. This hypothesis expanded on one formed a decade earlier when Evans (Evans and Norris 1993), after working in Brazilian waters, proposed that penaeid shrimp passively produce audible sound when swimming or feeding, and, when shrimp are congregated in great numbers, the sound is detectable.

Inspiration and indirect support for the earlier Evans hypothesis had come from passive sonar studies by Takemura (1972) on the influence of substrate, latitude, water depth, and distance from shore on the distribution and frequency of biological marine “frying” noises in the coastal waters of Japan. These waters are inhabited by penaeids, and the results revealed that the noise was absent when changes in any of the study variables created conditions that were either incompatible with penaeid habitat preference or outside the range of penaeid tolerance limits.

The Evans hypotheses led to the idea that passive sonar might be useful as a tool for the detection of shrimp for both stock monitoring and commercial purposes. Also, with knowledge of the acoustic parameters of shrimp signals and ambient noise, detection distance could be calculated from detection threshold, and the boundaries of shrimp beds could be determined. Detection of shrimp and definition of bed boundaries using passive sonar would eliminate the present search method of random trawling, thus decreasing the incidence of unnecessary by-catch and habitat disturbance, and possibly, increasing CPUE. Furthermore, if a relationship could be shown between signal production and the number of shrimp, then algorithms might be written for real-time computation of wild shrimp population abundance. Such a system could be used by shrimpers to assess the population of a bed before expending effort, thus increasing CPUE, and by government agencies and biologists for monitoring shrimp abundance and determining if and when shrimping closure should occur. This study initiated testing of the Evans hypotheses and, based on those results, suggested two possible methods for density calculation.

To test the hypotheses that Gulf of Mexico shrimp fisheries species make detectable sound, and that the sound is mechanically produced during normal shrimp behaviors, sound production by white shrimp (*Penaeus setiferus*) was studied acoustically and behaviorally. Video and audio recordings were made of aquarium-kept white shrimp. Audio recordings were made of wild populations in the bays and channels of Galveston, Texas, and simultaneously taken trawl data were used to verify species. Another crustacean-like signal from the Gulf of Mexico also was studied.

Data from "GulfCet" recordings made in the Gulf of Mexico of the unknown signal were analyzed and compared to white shrimp signals. All signals were characterized spectrally with the following results.

Captive white shrimp made detectable sound in association with three behaviors: eating, escaping, and burrowing. Trawl data confirmed that white shrimp were the harvested species. Comparisons between spectra of wild and captive shrimp signals eliminated burrowing and escaping as possibilities for the source of the wild signals. However, the signals produced by captive shrimp in association with eating were similar to the wild signals. Both captive eating and wild signals were impulsive transient "pops," typically 40-ms long, with energy concentrated from 2 to 8 kHz (Figs. 1–3). A sound pressure level (SPL)/source level (SL) of 112 dB (re: 1 μ Pa at 1 m) was calculated using the captive data. Wild spectra and captive eating spectra were highly correlated ($r = 0.7$ to 0.8 , $p < .05$). In conjunction with the supporting trawl data, the acoustic analysis suggested that the signals were produced by the same species engaged in the same activity (Fig. 4). Although the exact mechanism that produces the sound was not determined, the analysis supported the hypotheses that white shrimp, and probably all penaeids, in the Gulf of Mexico produce consistent detectable sound and that the sound is associated with eating. Because these sounds were easily detected and recorded in the wild, the use of passive sonar as a tool for remote detection of shrimp also was supported.

The wild signals, believed to be produced by white shrimp while eating, and the unknown crustacean-like signals from "GulfCet" data also were compared. Smoothed waveforms and acoustic envelopes were similar ($r = 0.9$) (Figs. 5, 6). The unknown signal had a slightly narrower bandwidth than the wild signal at 3 dB down of the magnitude spectrum squared (2.42 kHz

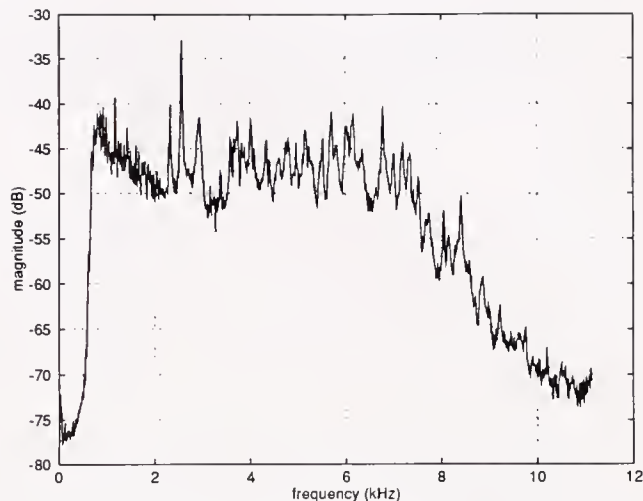


Figure 2. An averaged spectrum of eating signals recorded during feeding of white shrimp (*Penaeus setiferus*) in a glass aquarium and a fiberglass tank at Texas A&M University, College Station, and Galveston, Texas, respectively. Peak energy is at 2.3 kHz.

vs. 3.5 kHz, respectively) (Fig. 7). Water depth and geographic location suggested that the unknown signal might be produced by royal red shrimp, but verification of the species was not possible. Nevertheless, the data add further support for the use of passive sonar as a tool for remote detection of fisheries shrimp.

Based on these results, remote assessment of shrimp population densities may be possible using either: (1) the number of signals/time/area; or (2) amplitude changes/area. Signals are estimated to be produced at rate of 1/s to 2/s for an individual shrimp, thus, counting signals may be appropriate, especially where densities are

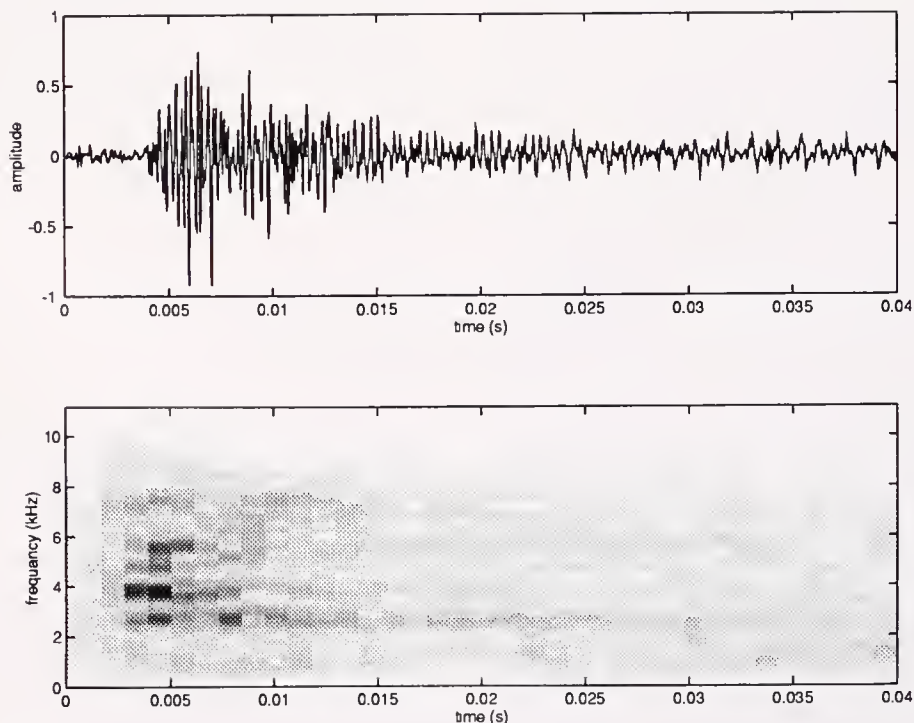


Figure 1. The oscillogram (top), and spectrogram (bottom), from a signal recorded during eating by captive white shrimp (*Penaeus setiferus*). The signal is an impulsive "pop," typically about 0.04 s long with energy concentrated below 8 kHz and a somewhat variable time-frequency structure.

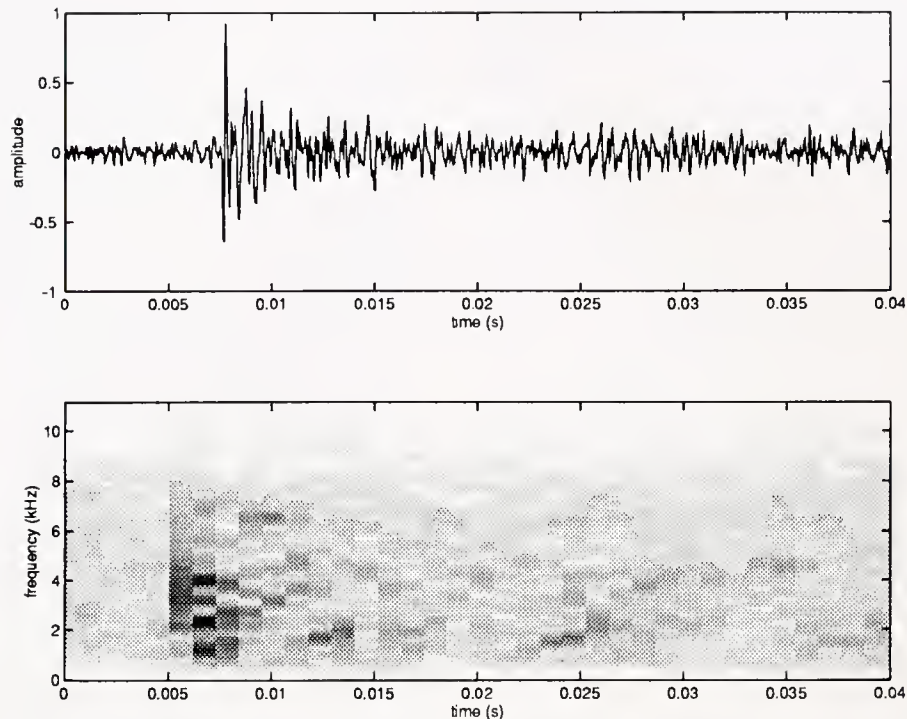


Figure 3. An oscillogram (top) and spectrogram (bottom) from a transient impulse recorded in the Galveston Ship Channel. The signals are structurally similar to eating signals of captive white shrimp (*Penaeus setiferus*).

low. However, in the event of very dense populations where many shrimp may be producing signals simultaneously, it may be necessary to use over-all amplitude changes to calculate density. Continued research was suggested using either passive acoustic modeling of mariculture pond populations followed by testing for goodness-of-fit to wild populations, or direct passive acoustic modeling of wild population densities (employing towed hydrophone array capabilities for calculation of the seafloor area from which signals are being received) with comparisons made to traditional trawl data density computations.

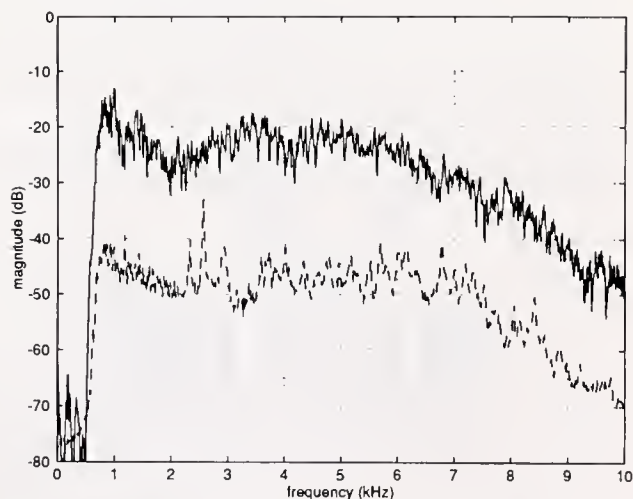


Figure 4. An averaged spectrum of transient impulses (solid line) recorded in the Houston Ship Channel compared to an averaged spectrum of eating signals (broken line) made by captive white shrimp (*Penaeus setiferus*) in a glass aquarium.

Since the presentation of this material at the Workshop on Spatial Data and Remote Sensing in Invertebrate Fisheries, density calculation research using the mariculture environment has begun in a joint venture between Texas A&M University and Auburn University. While at the workshop, this researcher was made aware that improved density calculation of shrimp is needed in the mariculture industry in addition to the need in the fishing industry. Shrimp ponds are stocked and harvested with known densities, but

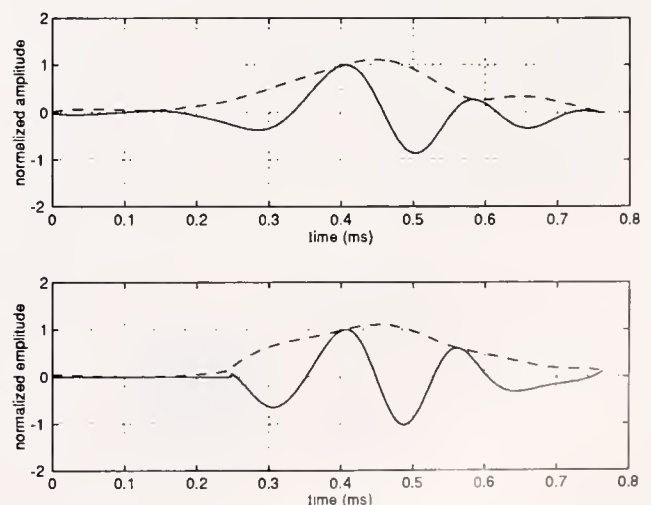


Figure 5. The waveforms (solid lines) and acoustic envelopes (broken lines) of impulsive transient signals associated with eating by shallow-water white shrimp (*Penaeus setiferus*) (top) and those of another crustacean-like signal (bottom) from deep water on the continental shelf in the Gulf of Mexico.

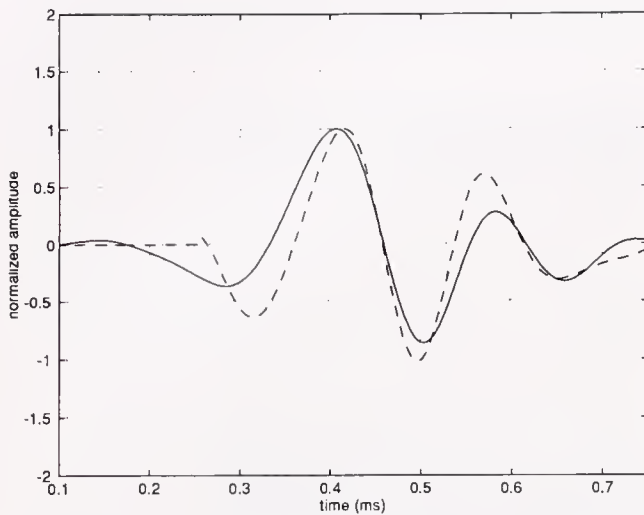


Figure 6. A comparison of the wave forms of white shrimp (*Penaeus setiferus*) eating signals (solid line) and an unknown crustacean-like signal (broken line). Note that the signals appear to be scaled.

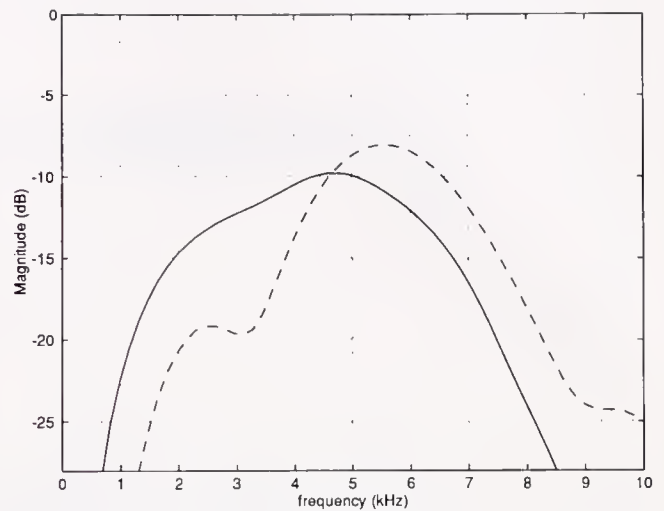


Figure 7. A comparison of the magnitude spectra of white shrimp (*Penaeus setiferus*) eating signals (solid line) and an unknown crustacean-like signal (broken line).

population assessment with available systems during the interim period is only marginally effective ($SE \pm 30\%$ to 50%) (Rodgers, Rouse, and Teichert-Cottingham, pers. comm.). The goals of this project are to develop a better method of population assessment for shrimp mariculture, then, as aforementioned, to field test the computations for eventual transfer to the shrimp fishing industry. Analysis of preliminary data is encouraging. Additionally, deter-

mination of the health of shrimp mariculture ponds also will be studied using passive acoustics and artificially induced changes in water chemistry. It is hypothesized that shrimp eating behavior will change in stress situations, thus, the rate of acoustic signaling should change. These differences will be used in conjunction with density data with the goal of using passive sonar as a tool for predicting and preventing shrimp mariculture pond crashes.

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Proceedings of a Special Session on

SEA URCHIN AQUACULTURE: MOLECULES TO MARKETS

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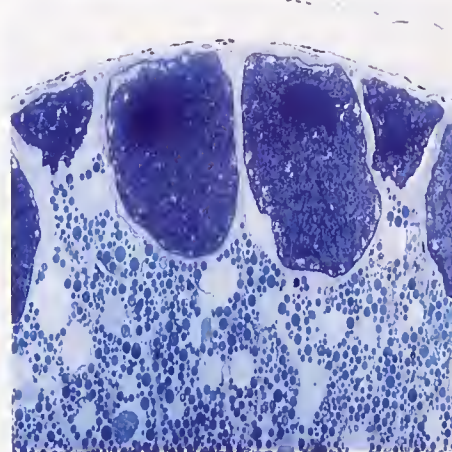
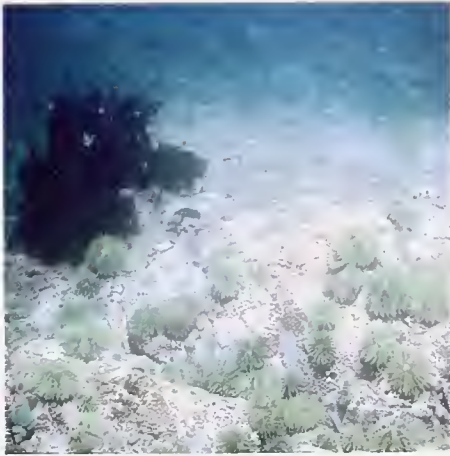
Michael P. Lesser and Charles W. Walker

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Top Left: Green sea urchins, *Strongylocentrotus droebachiensis*, in urchin harrens off the coast of New Hampshire. (M.P. Lesser)

Top Right: Green sea urchins in a large scale, land-based aquaculture system, University of New Hampshire Coastal Marine Laboratory. (C. Williams)

Middle Left: Gonads of the green sea urchin. (N.A. McGinn)

Middle Right: Plastic histological section of developing female gonad in *S. droebachiensis*. (N.A. McGinn and C.W. Walker)

Bottom Left: Sea urchin roe from Maine at the Tokyo Fish Auction. (Acadia Seafood International)

Bottom Right: Sea urchin roe from Japan at the Tokyo Fish Auction. (Acadia Seafood International)

INTRODUCTION TO THE SPECIAL SECTION ON SEA URCHIN AQUACULTURE

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KEY WORDS: Sea urchin, gametogenesis, gonad index, aquaculture

INTRODUCTION

In the early 1970's on the western coast of the United States, a lucrative fishery was developed for the red sea urchin, *Strongylocentrotus franciscanus* (Kato 1972). In the absence of fishing regulations, the size of individuals and the total catch of red sea urchins declined in the early 1980's (Kato and Schroeter 1985). Based on the analysis of data on gonad size, regulations were introduced in 1989 to limit the seasonal harvest of urchins and to establish a minimum harvestable size for sea urchins. These regulations have had limited success maintaining resource stability. As a result, the California fishery supports far fewer urchin harvesters today.

In the last 15 years, especially since 1987, a fishery for the green sea urchin, *Strongylocentrotus droebachiensis*, has become the seventh largest in the Northeastern United States, and is the third largest in Maine behind lobsters and aquacultured Atlantic salmon (NOAA NR96-11, September, 17, 1996). Early unregulated harvesting and continued overfishing have drastically depleted once abundant field populations in the Gulf of Maine. In Maine during 1993, 41 million pounds of urchins were harvested and 26 million dollars were earned by the urchin industry. Since this peak harvest, urchin landings have quickly declined while the price per pound has increased (Table 1). Recently, urchin fishermen, processors, and state government agencies in New Hampshire, Maine, and Massachusetts have recognized that a significant decline has occurred in urchin populations and have attempted to coordinate their efforts to realize a sustainable urchin harvest. Substantial data are available on seasonal changes in gonad size of Gulf of Maine green sea urchins and other populations around the world (Miller and Bishop 1973, Fletcher et al. 1974, Mottet 1976, Himmelman 1978, Keats et al. 1983, Vadas et al. 1989, Munk 1992). Despite these studies, which clearly show when harvesting would yield the maximum product and dollar value, regulation has been slow to match the rate of growth of the fishery.

Unsexed whole sea urchins or their processed gonads are shipped primarily to Japanese markets as a popular form of sushi called "uni" (and to the markets of France, Belgium, Greece, Italy, and Turkey). Several important, biologically based problems affect the large scale natural harvest of sea urchins in temperate ecosystems. First, as natural stocks dwindle and demand remains constant or increases, the harvesting of smaller, poorly fed natural populations results in urchins with low quality roe. Second, large populations of urchins still exist in deeper waters (below 18 m). Harvesting of these populations by SCUBA is potentially more dangerous and impractical in mid-winter when urchin gonads are most valuable. These deep water populations of urchins are also smaller in test size and have poor quality gonads as a result of limited resources of algae, which is the preferred natural diet of the green

sea urchin (Keats et al. 1984, Lawrence 1975, Vadas 1977, Larson et al. 1980, Briscoe and Sebens 1988, Prince and LeBlanc 1992). Third, smaller natural populations reduce the reproductively effective population which subsequently effects fertilization success and recruitment of juveniles. Last, the normal single annual gametogenic cycle, characteristic of sea urchins, limits the season when gonads are commercially valuable.

The examples above represent an all too common situation of over exploitation of a natural resource based on the limited use or knowledge of the basic biology of the harvested species. What can we do at this point? Clearly, there is a lucrative market for sea urchin roe. Equally clear is that we cannot continue to exploit natural populations at the present non-sustainable rate. Some self-regulation may occur as a result of the collapse of Asian currencies that we are presently experiencing, but they will eventually recover and the pressure to provide sea urchin roe from natural populations will increase, even with the regulations in place at this time. Various forms of sea urchin aquaculture may provide high quality and high dollar value products in the absence of large annual harvests of natural populations. In order to initiate aquaculture endeavors we must know about the basic biology of the cultured species, including aspects of disease, food requirements, and in the case of the sea urchin, their reproductive biology. It has become abundantly clear that we do not know as much about the reproductive biology of urchins as might be expected considering the voluminous literature on urchin biology. A more complete understanding of the reproductive biology of urchins is needed to harvest natural populations on a sustainable basis or to initiate urchin aquaculture. The purpose of this special section on sea urchin aquaculture was to gather a group of biologists, aquaculturists, and economists with a common interest in the aquaculture or the sustainable harvest of natural populations of sea urchins. This group is not exhaustive in its scope and neither is the information presented here. Collectively, these papers should not be looked upon as the definitive

TABLE 1.

Landing statistics compiled by the Maine Department of Marine Resources for green sea urchin (*Strongylocentrotus droebachiensis*) landings through 1996.

Year	Pounds Landed	Average Price/lb.	Value (US Dollars)
1987	1,440,161	0.16	2,236,391
1988	6,221,604	0.28	1,758,805
1989	9,657,158	0.38	3,698,038
1990	13,227,430	0.45	5,995,975
1991	20,535,411	0.54	11,158,425
1992	26,502,068	0.58	15,426,363
1993	41,073,687	0.65	26,519,733
1994	38,166,941	0.86	32,803,694
1995	31,998,063	1.04	33,180,743
1996	22,000,000	1.09	24,000,000

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writings on the state of sea urchin aquaculture. They do represent many of the current directions in sea urchin aquaculture and as such point toward some areas of urchin biology for which we have little or no information. We begin with a short review on sea urchin gametogenesis by Walker et al. which provides some novel ideas about the use of molecular tools to monitor different stages of gametogenesis. This review is followed with an article by Lawrence and Bazhin on the utility of various species of sea urchins as appropriate candidates for aquaculture, based on their life-history characteristics. A paper by Grosjean et al. then describes the cultivation of urchins in a land-based aquaculture facility from larvae to market size. Two papers (Hagen and Agatsuma) deal with gonad yields in urchins when fed algal diets, while the majority of the papers deal with varying aspects of developing and using formulated feeds or polyculture to produce high quality gonads. Lastly, one paper (Kessing and Hall) reviews the status of

urchin populations around the world and the potential for new aquaculture opportunities.

We hope that reading this collection of papers will encourage collaborative efforts between the private sector, aquaculturists, and biologists interested in sea urchin aquaculture.

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NEW PERSPECTIVES ON SEA URCHIN GAMETOGENESIS AND THEIR RELEVANCE TO AQUACULTURE

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INTRODUCTION

Gametogenesis is a single annual event in both sexes of most species of sea urchins that live in higher latitudes. It involves an abrupt change in activity within the gonads from storage of nutrients in somatic cells to the rapid increase in numbers and/or size of germ cells (Pearse and Cameron 1991). The bulk of the nutrients utilized in the production of either ova or spermatozoa is initially stored in intragonadal somatic cells called nutritive phagocytes (NP). NP are the only obvious somatic cellular components of the germinal epithelia in both testes and ovaries (Holland and Giese 1965, Holland and Holland 1969). In some ways, NP function like the Sertoli cells of the mammalian testis (Hinsch 1993, Guraya 1995).

Most available information on gametogenesis in sea urchins is presented in ecological studies and is based on the assessment of gonad index (GI, the ratio of gonad mass to total body mass). Although histological studies of annual gametogenesis are available for several species of sea urchins, only a few consider cytology and ultrastructure during oogenesis (Verhey and Moyer 1967, Tsukahara and Sugiyama 1969, Tsukahara 1971, Landim and Beig 1979, Nicotra and Serafino 1988, Byrne and Siegel 1998) and even fewer during spermatogenesis (Longo and Anderson 1969). Very little data on the physiology or molecular biology of sea urchin gametogenesis are available. Chemical signaling mechanisms that stimulate or inhibit gametogenesis are simply uninvestigated in sea urchins. Changing photoperiod is often correlated with the initiation of gametogenesis in both sexes of some sea urchins (Pearse et al. 1986, Walker and Lesser 1998), although this is not always the case (Yamamoto et al. 1988, Sakairi et al. 1989, Ito et al. 1989). Existing molecular studies deal mostly with major yolk proteins found in the coelomic fluid or developing oocytes (Verhey and Moyer 1967, Tsukahara 1971, Harrington 1982, Ozaki 1982, Harrington and Ozaki 1986, Ozaki et al. 1986, Shyu et al. 1986, Shyu et al. 1987). This over-all lack of basic information on sea urchin gametogenesis is surprising in an organism that provides major models for studies of fertilization and early development and alarming in an animal that is often subject to intense commercial harvest.

In this paper, we combine classic and recent information to generate new models for gametogenesis in those sea urchins sensitive to photoperiod and that live in temperate waters. We also provide some examples of how gametogenesis can be manipulated to produce urchins with large gonads characterized by superior taste, color, texture, and firmness. We hope that this information will be useful to those interested in the biology and aquaculture of sea urchins.

THE CLASSICAL VIEW

Based on histological analysis of paraffin sections taken during the annual reproductive cycle of two species of sea urchins, Fuji (1960a, 1960b) recognized five stages for gametogenesis and Nicotra and Serafino (1988) recognized three stages for NP. Following spawning, the germinal epithelium contains shrunken and gonial stem cells (either oogonia or spermatogonia) (Fuji's *Spent Stage*; Nicotra and Serafino's NP *Resting Stage*). Nutritive phagocytes begin to grow during this period and store protein, lipid, and carbohydrate nutrients (Fuji's *Recovering Stage*; Nicotra and Serafino's NP *Growing Stage*). As gametogenesis begins, gonial cells increase in number around the bases of enlarged nutritive phagocytes (Fuji's *Growing Stage*). Gamete differentiation occurs as germinal cells progress through gametogenesis and move toward the luminal ends of nutritive phagocytes (Fuji's *Premature Stage*). During gametogenesis, nutritive phagocytes shrink and lose their nutrient reserves as primary oocytes grow to large size (65–150 μm) and as differentiated spermatozoa accumulate in the gonadal lumen (Fuji's *Mature Stage*; Nicotra and Serafino's NP *Depleting Stage*).

Versions of these stages have been used to characterize gametogenesis for sea urchins from around the world (Byrne 1990, Meidel and Schleibling 1998, Spirlet et al. 1998, Unuma et al. 1998). Although these staging systems are easy to use in following basic features of gametogenesis, detailed cellular events do not fit well in these contexts. For example, the differential growth of nutritive phagocytes and germinal cells is difficult to characterize using either of these staging systems alone. Such events as the initiation and completion of nutrient storage and the timing of oogonial and spermatogonial mitosis and differentiation also fit poorly into these staging systems. As more data become available, it is desirable to place them in a cellular and molecular context. The following sections on stereology, cytology, and molecular biology demonstrate the kinds of new data that are being generated and attempt to provide a more cellular and molecular framework for understanding sea urchin gametogenesis.

Gonad Index and Stereology

The gonads of sea urchins perform the dual function of storing nutrients and producing gametes (Giese 1966). Gonad index has traditionally been used to assess gametogenesis in sea urchins. However, GI alone does not discriminate between growth of nutritive phagocytes and the gametogenesis. A more useful technique to measure the growth of NP and germinal cells would provide a quantitative measure of the relative volumes of the gonad occupied by each of these cellular populations at any time during gameto-

genesis. Stereology is a quantitative technique that uses a mathematical approach and point-count analysis to obtain three-dimensional (3-D) information from two-dimensional (2-D) sections of sea urchin gonads (Elias and Hyde 1980, Walker and Lesser 1998). The data that result from this kind of analysis are also amenable to statistical testing of hypotheses related to environmental, physical, or molecular factors affecting changes in gonad size. For example, stereological, gonad index, and histological data from Walker and Lesser (1998) indicate that gonads grow to a larger size ($\geq 20\%$ GI) in urchins reared in the laboratory and supplied with a pelletized commercial food (Lawrence et al. 1997) than the gonads of urchins collected from the field ($\leq 15\%$ GI) (Fig. 1). Unusually abundant development of nutritive phagocytes was responsible for the increase in gonadal growth observed in animals in the laboratory (Fig. 1). In female urchins, a small or modest reduction in size of nutritive phagocytes after the initiation of oögonial mitosis suggests that nutrients were being transferred to oocytes for vitellogenesis. In male urchins, nutrients mobilized from storage in enlarged nutritive phagocytes were presumably utilized during normal spermatogenesis and were responsible for the large gonad indices after exposure to fall photoperiod ($\geq 25\%$, Walker and Lesser 1998). Because gonads containing only a few gametes have the highest commercial value, stereological data on the relative abundances of the NP and gametes at various stages of the reproductive cycle would be a very valuable parameter for aquaculturists to monitor.

Gametogenesis at High Resolution—Some New Insights

Other than the few studies previously mentioned, all histological examinations of gametogenesis in sea urchins are based on sections of paraffin-embedded gonadal samples. Paraffin sections are very useful for studying conspicuous changes within the gonad. However, the ability to discriminate carefully between different cell types and to observe cellular events such as mitosis and meiosis is often difficult within paraffin sections. The problem is intensified by the small size of echinoderm cells (e.g., oögonia and spermatogonia range between 5 and 10 μm in diameter) and the lack of resolution of standard paraffin histology. The increased resolution available in sections of plastic embedded sea urchin

gonads reveals considerable cytological detail not evident in paraffin sections. For example, it is possible: (1) to observe details of germinal cells undergoing various stages of mitosis and meiosis; (2) to determine the boundaries of individual nutritive phagocytes; and (3) to monitor changes in the composition of granules within the cytoplasm of primary oocytes and of the nutritive phagocytes.

In the following, we present data derived from an ongoing annual study of gametogenesis in *Strongylocentrotus droebachiensis*. This study is based on sections of plastic-embedded samples of ovaries and testes collected monthly ($n = 20$) during the annual reproductive cycle. In these samples, we have observed four distinct gametogenic stages (Fig. 2) characterized by major features of the germinal epithelium, the nutritive phagocytes, and the germinal cells.

NP Renewal and Pregametogenesis Stage

This stage is characterized by the growth of the nutritive phagocytes and by the accumulation of granules of uniform size and shape in their cytoplasm (Fig. 3a, 4a). These granules consist of carbohydrates, proteins, and lipids. The NP typically reach maximum size toward the end of this stage. Late in this stage, clusters of amitotic oögonia are apparent near the bases of the NP (Fig. 3a). Primary oocytes that appear oval or triangular in sections and are located between the NP probably remain from the previous year's oögenesis. In the testes during the later months of this stage, an increase in the numbers of spermatogonia is noticeable near the bases of the NP (Fig. 4a). Mitotic divisions of spermatogonia were also observed earlier in the year, during the summer (June, July), indicating that spermatogenesis can be initiated long before oögenesis begins. Anecdotal observations on a variety of sea urchins also suggest that spermatozoa can be produced before the initiation of oögenesis (Dr. John Lawrence pers. comm.).

NP Utilization and Gametogenesis Stage

Throughout this stage the NP are mobilizing nutrients, and the granules within them are losing their uniformity (Fig. 3b, 4b). In

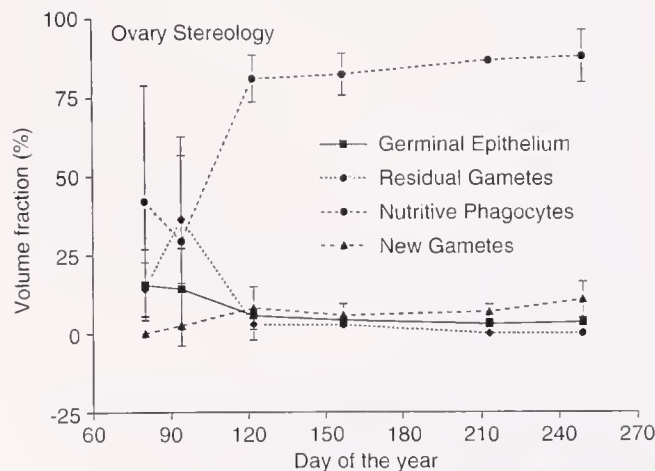


Figure 1. *Strongylocentrotus droebachiensis*—The volume fraction (V_v) of germinal epithelium, nutritive phagocytes, residual gametes, and new gametes relative to gonad tubule volume for monthly samples of female urchins. (with permission, *Mar. Biol.*, Walker and Lesser, 1998).

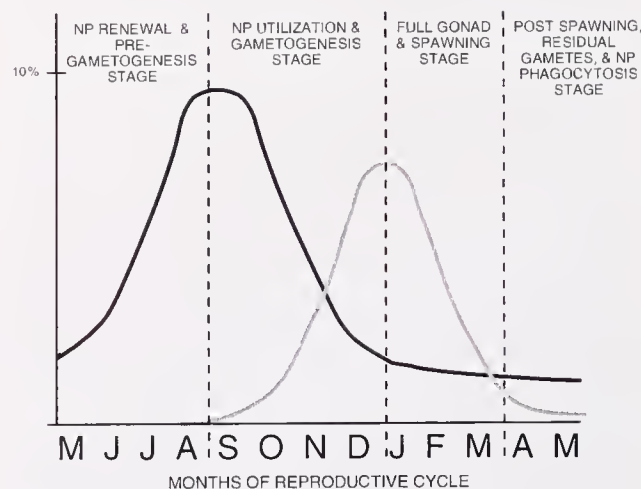


Figure 2. Representation of stages of the gametogenic cycle of sea urchins over 1 year (May to May): NP renewal and pregametogenesis; NP utilization and gametogenesis; full gonad and spawning; post-spawning, residual gametes, and NP phagocytosis. The curves represent the changes in estimated abundance of nutritive phagocytes (dark curve) and gametes in the gonads of either sex (light curve), based on qualitative observations of typical urchins from each stage.

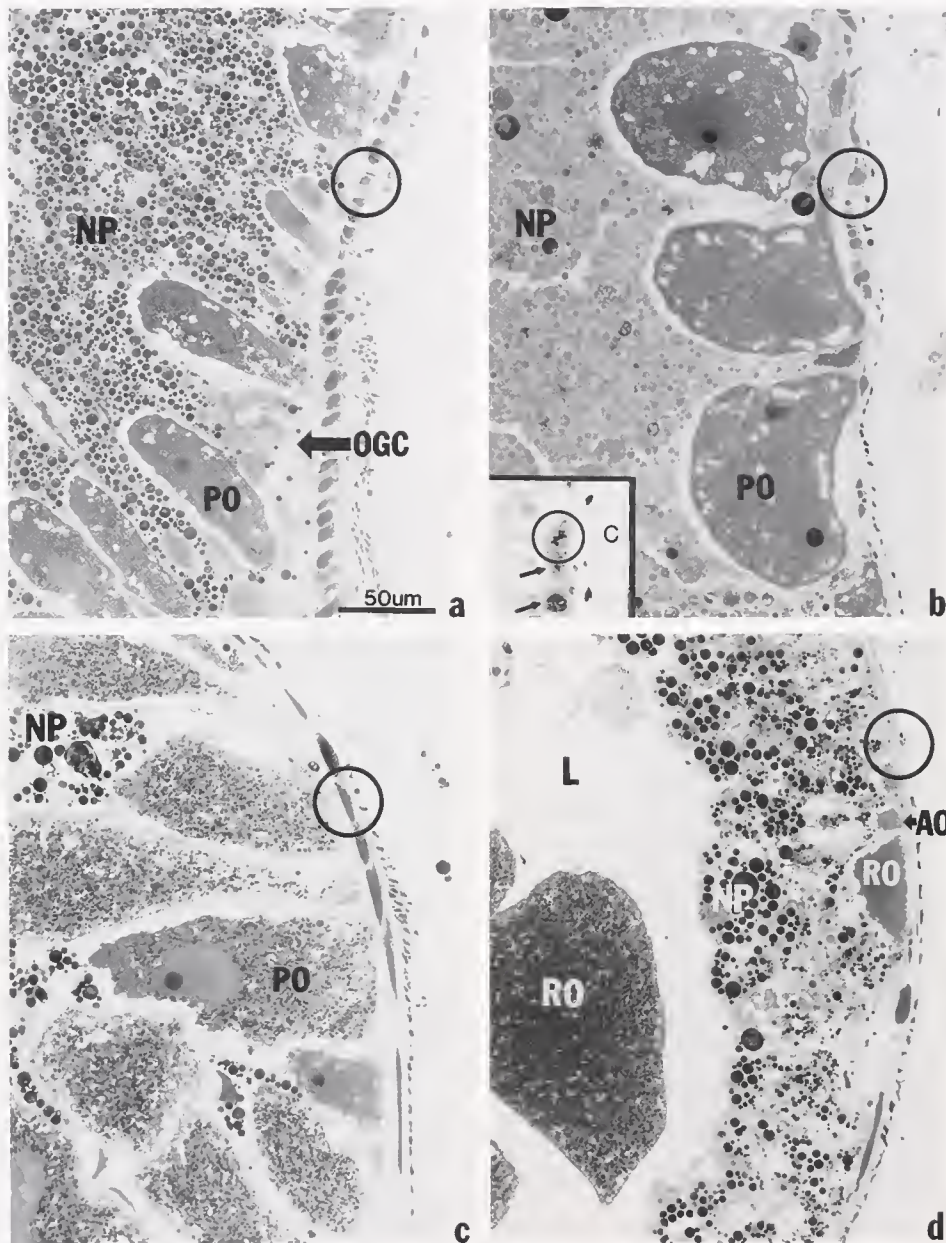


Figure 3. *Strongylocentrotus droebachiensis*—Plastic sections of ovaries from a) NP renewal and pregametogenesis; b) NP utilization and gametogenesis; c) full ovary and spawning; d) postspawning, residual gametes, and NP phagocytosis stages of the annual oogenic cycle. AO, amitotic oögonia; L, ovarian lumen; NP, nutritive phagocyte; OGC, oogonial cluster; PO, primary oocyte; RO, residual oocyte or ovum. Circle indicates the ovarian wall. Scale bar for all figures, 50 μ m. Inset, oogonial mitosis in circle; C, coelom. (inset with permission from *Mar. Biol.*, Walker and Lesser, 1998).

ovaries, oogonial mitosis has concluded (see inset of an oogonial mitosis), and major growth of primary oocytes occurs (Fig. 3b). A germinal vesicle containing a large nucleolus is visible within the oocytes, reflecting the high levels of rRNA synthesis occurring during this stage (Cowden 1996). In testes, numerous mitotic divisions of spermatogonia and meiotic divisions of spermatocytes are evident (Fig. 4b; see inset of a spermatogonial mitosis). Differentiated spermatozoa are obvious in the testicular lumen.

Full Gonad and Spawning Stage

At this stage, there is a further reduction in size of the NP, and the granules remaining within them are few and nonuniform in size

and contents (Fig. 3c, 4c). Both NP and large, granule-filled oocytes extend on thin stalks of cytoplasm from the gonadal wall toward the ovarian lumen (Fig. 3c). In testes, the lumen is full of differentiated spermatozoa ready for release, and spermatogonial mitosis is limited (Fig. 4c). Spawning of gametes in both sexes of *S. droebachiensis* occurs toward the end this stage in the late winter/early spring.

Postspawning, Residual Gametes, and NP Phagocytosis Stage

In this stage, small NP differ greatly in appearance from earlier stages and contain empty, membrane-bound vacuoles and few or no granules (Figs. 3d, 4d). In ovaries, residual oocytes or ova

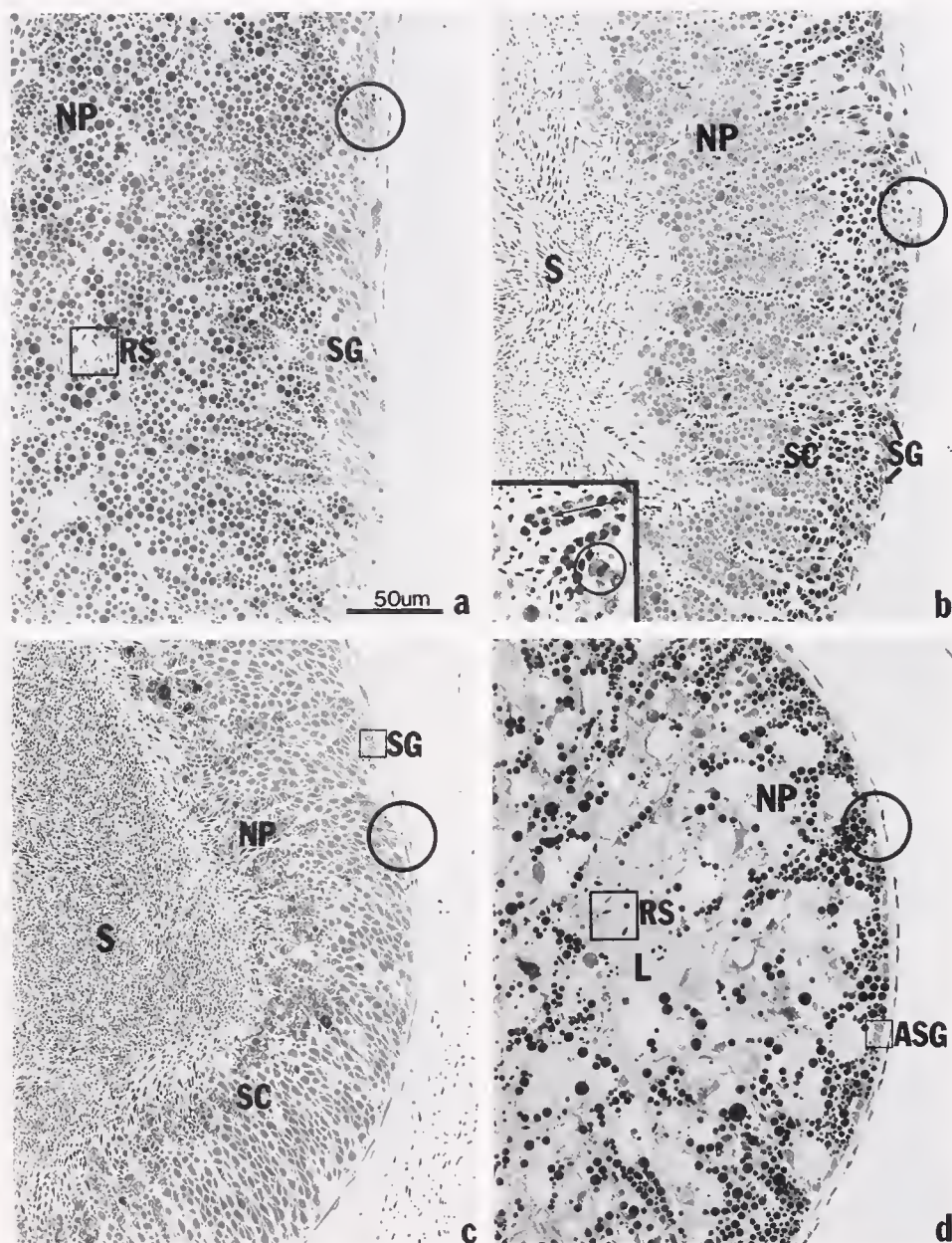


Figure 4. *Strongylocentrotus droebachiensis*—Plastic sections of testes from a) NP renewal and pregametogenesis; b) NP utilization and gametogenesis; c) full testis and spawning; d) postspawning, residual gametes, and NP phagocytosis stages of the annual spermatogenic cycle. ASG, amitotic spermatogonia; L, testicular lumen; NP, nutritive phagocyte; SG, spermatogonia; SC, spermatocyte; RO, residual spermatocyte. Circle indicates the testicular wall. Scale bar for all figures, 50 µm. Inset, spermatogonial mitosis in circle. (inset with permission from *Mar. Biol.*, Walker and Lesser, 1998).

(containing obvious cortical granules near the cell membrane) are found in the lumen and between the NP. Single, amitotic oögonia are found around the bases of the NP (Fig. 3d). In the testis, residual spermatozoa remain in the lumen, and amitotic spermatogonia are apparent as individual cells at the bases of the NP (Fig. 4d). In the gonads of both sexes, residual gametes are phagocytized by NP during this time. Residual gametes are often observed in the early months of the next stage (= NP renewal and pregametogenesis stage).

MOLECULAR BIOLOGY, BIOCHEMISTRY, AND GAMETOGENESIS

Molecular studies of sea urchins are almost exclusively limited to housekeeping genes and to those involved in larval develop-

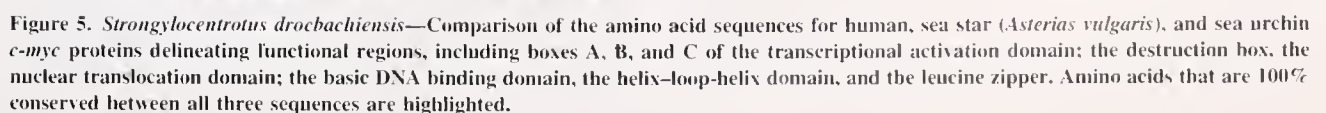
ment. An understanding of the patterns of expression of genes related to the growth and metabolism of NP and of gametes would be useful. When considered along with stereology and high-resolution plastic sections, the expression of key genes could be used to discern the differential activities of NP or gametes during gametogenesis. Study of appropriate genes and of their protein products should also provide easy to follow indicators of the overlapping processes of NP growth and gametogenesis. Northern and Western analysis of the expression levels for RNA and protein for these genes could be accomplished more quickly than labor-intensive stereology and histology.

Some genes have been cloned from sea urchins and are indicative of the activities of the NP and the gametogenic cells. In particular, the cDNA sequences for a major yolk protein (MYP)

is also lost from nutritive phagocytes, but is not identifiable by the antibody as a component of germ cells at any stage of spermatogenesis (Unuma et al. 1998). The ability to follow this major component of the yolk is an exciting application of molecular biology to the understanding of sea urchin gametogenesis. Until now it has not been possible to prove that nutrients mobilized from the NP are actually transferred to the oocytes. The fate of the identical protein in males is still enigmatic and deserves further study.

C-myc—An Indicator of Gonial Cell Mitosis and of the Length of the Gametogenic Cycle

The molecular signaling pathways that lead from the environmental cue of photoperiod to the initiation of gametogenesis in sea urchins are virtually uninvestigated. Nonetheless, molecular data that may be relevant are available for sea urchins. In animal somatic cells, a common denominator in both steroid- and protein-based mitogenic pathways is the expression of the transcription factor, *c-myc* (Henriksson and Lüscher 1996). The *c-myc* protein activates the expression of downstream target genes relevant to the cell cycle (Bello-Fernandez et al. 1993, Rosenwald et al. 1993, Roy et al. 1994), and its activity is an essential precursor to cellular mitosis. A *c-myc* gene has been cloned from the sea urchin (GenBank Accession # L37056). This *c-myc* cDNA is highly similar to the sequences in the sea star, *Asterias vulgaris* (Walker et al. 1992), and the human. Based on its structure, *c-myc* protein should act in a similar way during the cell cycle of sea urchins, sea stars, and humans (Fig. 5). Therefore, expression of *c-myc* is an early, detectable indicator of mitosis in sea urchin gonial cells (= the beginning of gametogenesis). It should indicate exactly when gonial cell mitoses occur and would also demonstrate the length of time gonial cells spend proliferating during the reproductive cycle.



These data are available only as nonquantitative measurements of histological sections of sea urchin testes and are completely unknown for sea urchin ovaries. A simple Northern screening of monthly samples of RNA from sea urchin gonads would be very informative. Gonads with relatively few gametes are most desirable to the industry. It would be possible to monitor sea urchins in the field or in an aquaculture facility for the expression of *c-myc*. If such analysis indicated that expression of *c-myc* had commenced, then the crop of urchins should be harvested before the gonads carry out extensive gametogenesis and lose their firmness.

Carotenoids—Indicators of the Color Quality of the Gonads of Sea Urchins

In its present configuration, the Lawrence pelletized food (Lawrence et al. 1997) contains β carotene, which gives a satisfactory, but not ideal, color to the gonads of *S. droebachiensis*. It is desirable to improve this color by using a different carotenoid supplement in the food. Females of *S. droebachiensis* metabolize β carotene into echinenone (4-keto- β -carotene), which accounts for 80% of carotenoids in the ripe ovaries (Griffiths and Perrott 1976). Animals with the darker yellow orange colored gonads that are desirable to the commercial market contain a high percentage of this carotenoid. In the future, we intend to have the diet synthesized to contain echinenone. This should finally resolve the problem of a less than desirable gonad color that results from using the current formulation of the pelletized food. It is also important to assess the carotenoids present in the mature testes of *S. droebachiensis* in order to accomplish this same result.

MODELS FOR SEA URCHIN OOGENESIS AND SPERMATOGENESIS—NEW INSIGHTS

By combining classic and new data such as those described above, we have developed the following models for sea urchin gametogenesis in species such as *S. droebachiensis*, which are photoperiod sensitive. Previous staging systems by Fuji (1960a, 1960b), Nicotra and Serafino (1988), and Walker (1982) are also included to permit the use of data available on many other sea urchins. The models are not exhaustive and merely suggest a new, more cellular context for understanding gametogenesis in sea urchins. Certainly, as more information becomes available, it will be necessary to modify these models. With some adjustment, these models may also work for other sea urchins that have multiple periods of gametogenesis during the year, such as those in tropical waters.

Oogenesis (based on S. droebachiensis)

A model for annual oogenesis in the sea urchin is depicted in Figure 6a. This model begins by illustrating the condition of the germinal epithelium in an ovary in late winter and early spring. Such ovaries contain amitotic oögonia, residual oocytes or ova, and nutrient depleted, phagocytic NP. In April or June, storage of nutrients (lipid, carbohydrate, and protein) begins (Himmelman 1986), and NP grow substantially until August or September. The bases of the NP are surrounded by clusters of amitotic oögonia. In the fall, changing environmental photoperiod leads to shorter days. There follows a major mobilization of nutrients from NP and a simultaneous initiation of oögonial mitosis observable in plastic sections. It is unclear whether the photoperiod cue directly stimulates oögonial mitosis or whether it is indirectly stimulated as a result of the nutrients available from the NP. Perhaps the photo-

period cue directly stimulates nutrient mobilization from the NP. It is clear that the major yolk protein that composes most of the protein stored in the NP is transferred to primary oocytes as they undergo vitellogenesis. NP shrink as primary oocytes grow. Finally, the ovarian lumen is filled with stored primary oocytes that have attained maximum size (≈ 130 – 160 μ m in diameter). Primary oocytes undergo meiosis and become fully mature ova during ovulation. Within the ovary, residual primary oocytes (lacking a germinal vesicle) or ova (containing cortical granules near the oocyte membrane) persist as the oögonic cycle begins again.

Spermatogenesis (based on S. droebachiensis)

Figure 6b illustrates the more complex annual spermatogenic cycle of the green sea urchin. At the right of this figure is the germinal epithelium of a testis following spawning. It contains nutrient-depleted NP, residual spermatozoa, and clusters of amitotic spermatogonia. During growth (and perhaps increase in the number) of the NP and storage of nutrients in the summer months, limited initiation of spermatogonial mitosis and minor sperm production can occur. This phenomenon has not previously been mentioned in the literature, although anecdotal information indicates that it may be fairly common (Dr. John Lawrence). It is possible that nutrients sufficient to support local spermatogonial mitosis and subsequent spermatogenesis leak from the large NP as they grow. During such times, males can respond by mitotic division of spermatogonia during the summer (June–August) and even by the production of spermatozoa. This was never observed in females, where the production of an oocyte with a diameter of ≥ 130 μ m simply requires more nutrients. As NP reach maximum size and fall photoperiod commences, major mobilization of nutrients from NP and extensive spermatogonial mitoses follow. Subsequent events of spermatogenesis produce differentiated spermatozoa that are stored in the testicular lumen. Upon release, spermatozoa are activated in sea water.

SEA URCHIN GAMETOGENESIS AND AQUACULTURE

It is obviously not practical for the sea urchin industry alone to take on the added effort associated with stereology, high-resolution histology, and molecular biology. The expertise and the infrastructure for these techniques already exists within the academic community. As a result, we strongly encourage the urchin industry and interested biologists with knowledge of these approaches to join in collaborative basic and applied research. Together, they can continue to provide a better understanding of the reproductive biology of the sea urchin and also suggest how to apply this knowledge in producing urchins that contain large gonads characterized by superior taste, color, texture, and firmness. We have provided a modern framework for the acquisition and organization of cellular, physiological, and molecular data on the biology of sea urchin gametogenesis. Creative development and application of this knowledge can lead to mechanisms for manipulating the gametogenic process to yield a high quality aquaculture product.

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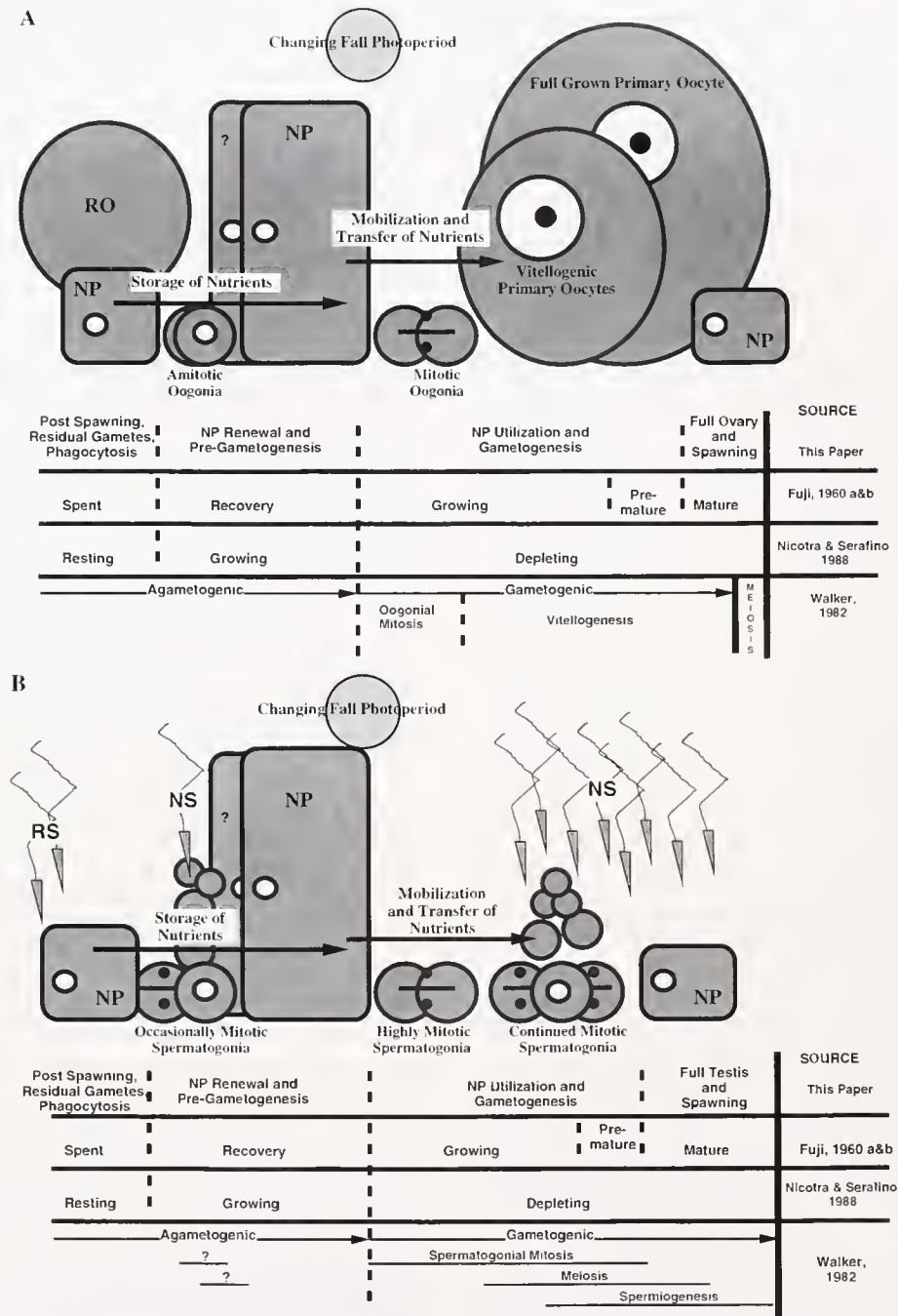


Figure 6. a) Model for the annual oogenic cycle of sea urchins. NP, nutritive phagocyte; RO, residual oocytes or ova; b) model for the annual spermatogenic cycle of sea urchins. NP, nutritive phagocyte; NS, new spermatozoa; RS, residual spermatozoa.

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LIFE-HISTORY STRATEGIES AND THE POTENTIAL OF SEA URCHINS FOR AQUACULTURE

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ABSTRACT Life history characteristics of sea urchins can be used to evaluate the potential of species for aquaculture. The important life history characteristics for this evaluation can be separated into those affecting production of biomass and maintenance. These result from three primary life history strategies. First, species in environments that are unstable or with high predation and with high food availability should have high production and low maintenance. Second, high production and moderate maintenance and a potential for phenotypic plasticity should occur in species in environments that are stable with low predation and high food availability. Third, those in environments that are stable or with low predation and with low food availability should have low production and high maintenance. *Triplonectes*, *Strongylocentrotus*, and *Arbacia* are probable representative genera, respectively, of these life history strategies. Species in the first category would be most appropriate for aquaculture using production as the criterion. The consumption by humans of certain species of sea urchins in the past can be related to their accessibility and palatability, and does not necessarily indicate their suitability for aquaculture. Knowledge of life history characters can aid in evaluating sea-urchin species for aquaculture.

KEY WORDS: Aquaculture, sea urchins, life history strategies, life history characteristics

INTRODUCTION

Dialogue (Diálogo) Santiago de Chile, 1997

José: "John, are sea urchins eaten in the United States?"
("¿Juan: los erizos de mar se comen en los Estados Unidos?")

John: "No." ("No.")

José: "No! Why not?" ("¿No! ¿Porqué no?")

John: "No reason." ("Sin ninguna razón.")

John: "José, is the black sea-urchin eaten in Chile?"
("¿José: se come el erizo negro en Chile?")

José: "No." ("No.")

John: "No! Why not? There are lots of them."
("¿No! ¿Porqué no? Hay muchos.")

José: "No reason." ("Sin ninguna razón.")

Alberto Larrain (Universidad de Concepción) has a reason why the black sea-urchin *Tetrapygus niger* (el erizo negro) is not eaten in Chile, and it is not a cultural reason as in the United States. He suggests *T. niger* would be eaten if it were palatable because it is abundant (Vásquez and Buschmann 1997). After repeated questioning of Chileans, one person was found who had spoken to someone who had eaten the roe of *T. niger* and three persons who had actually done so. The verdict in all cases was that the roe of *T. niger* tastes bad. Similarly *Arbacia lixula*, in the same arbacioid family as *T. niger*, is eaten only locally in Mediterranean countries; whereas, *Paracentrotus lividus* (l'oursin comestible) is widely eaten there (Le Direac'h et al. 1987). *Arbacia lixula* is considered the "father urchin", and *Paracentrotus lividus*, the "mother urchin." But why should the eggs of arbacioids not only taste bad, but have a dark violet color in contrast to the usual yellow or orange of sea-urchin eggs like those of commercial species such as *P. lividus* and the strongylocentrotids? An unusual color can be an aposematic signal indicating the presence of a secondary metabolite, warning potential predators of an unpalatable or toxic prey (Harvey and Greenwood 1978). Here the warning would be of the

spawned egg, free in the water column and vulnerable to predation. But why should arbacioids have this metabolic expense of producing a secondary metabolite that *P. lividus* and the strongylocentrotids do not? Can it be associated with a life-history strategy? And if so, can life-history strategies be important in evaluating the potential of species of sea urchins for aquaculture? Do life-history strategies have a basis in the energy requirements and usages that can be useful in understanding the characteristics of species necessary for evaluating their potential? Here we relate energy budgets to life-history strategies and suggest they can be used for this purpose.

Energy Budgets

According to the second law of thermodynamics, living systems must use energy to maintain themselves over time (von Bertalanffy 1952). As open systems, organisms do this by obtaining energy from the environment and allocating it to various activities. The relation between energy obtained and its allocation is expressed in the energy-budget equation (Ricker 1968):

$$C - F = A = R + U + P_r + P_s$$

where C is consumption, F is feces, A is absorption, R is respiration, U is excreta, P_r is reproductive production, and P_s is somatic production. The energy associated with respiration is for both anabolism and maintenance. P_s can be for primary structures or secondary structures for defense. P_r and P_s together constitute total production or assimilation. Energy budgets require use of energy units (Joules). Other units (mass) can be useful for other purposes, but only energy units allow calculation of the relative, not absolute, allocation to the different functions that is essential to establishing life-history strategies (Calow and Townsend 1981). Complete energy budgets are necessary for internal consistency. Few energy budgets have been established for sea urchins, and these are not always complete. Fuji's (1967) energy budget for *Strongylocentrotus intermedius* lacks the respiration term and Propp's (1977) energy budget for *Strongylocentrotus droebachiensis* lacks the consumption term.

Life-History Strategies

That selection can be linked to energy budgets underlies the concept of life-history strategies (Ebert 1982). This results from the trade-offs in the energy budget that can occur between somatic growth, production for gonadal growth, and survival so that allocation to one function is not available for others (Calow 1984). Life-history strategies consider how these characteristics vary in a coordinated evolution to increase fitness (Stearns 1992). The interaction of these characteristics is apparent in the Euler-Lotka equation (Calow 1984):

$$1 = \sum e^{-rt} s_t n_t$$

where r is the Malthusian parameter (intrinsic rate of natural increase); t is time to maturity, a function of somatic production; s is survival, a function of maintenance and protection; and n is fecundity, a function of gonad production. Fitness is increased by increasing s and n , and by decreasing t .

Grime (1977) related life-history strategies to two external factors that limit energy in an individual. One, stress, restricts production of biomass. It includes low levels of food, capacity to feed, and suboptimal abiotic conditions. The other, disturbance, results in the loss of biomass. This is usually lethal for animals resulting from biotic (predation) or abiotic factors. Species that experience high disturbance, *ruderal species*, would be expected to have low survivorship (s), a high growth rate (a short time to maturity t), and a high fecundity (n). These species would put little energy into maintenance or protection, because it is allocated to growth and reproduction. Species that experience high stress, *stress-tolerant species*, would have a low fecundity, a slow growth rate, and high survivorship. These species would put much energy into maintenance or protection, because it is not allocated to growth and reproduction. This increases the probability these species survive long enough to reproduce successfully. Species that experience low disturbance and low stress, *competitive species*, allocate energy to all functions. These three primary strategies should be

found at the extreme levels of stress and disturbance. Secondary strategies should be found at intermediate levels.

Life-History Strategies and the Potential of Sea Urchin Species for Aquaculture

Using production alone as the criterion, the most appropriate sea urchin species for aquaculture should be those that have the shortest time to maturity (high growth rate), highest fecundity, and lowest survivorship. They should allocate little energy to maintenance or protection. Those species that do allocate energy to maintenance may be better able to withstand stress. Lawrence (1990) assigned sea urchin species to specific life-history strategies according to their characteristics. The question here is whether these characteristics can be used to evaluate the potential of sea urchins for aquaculture.

Is interest in aquaculture of particular species because they are appropriate or because they are available? Certainly, availability is essential, but the examples of *Tetrapygus niger* and *Arbacia lixula* indicates this is not sufficient. Different life-history characteristics (tactics) can be predicted to be associated with the different strategies of sea urchins (Table 1). Species should have a suite of related characteristics appropriate for a particular life-history strategy. Thus, a species such as *Tripneustes ventricosus* that lives in a habitat subject to high disturbance but with high food availability would be predicted to have a rapid growth rate, high reproductive effort, high metabolism, low maintenance, and a short life span. We consider whether these characteristics indicate the appropriateness of sea urchin species for aquaculture.

Growth Rate

Ebert (1975, 1982) separated sea urchin species according to their rates of growth, concluding that species such as *Strongylocentrotus droebachiensis* are more slowly growing than *Tripneustes ventricosus* and *Lytechinus variegatus*. A comparison of species can be made best if individuals are fed the same food under

TABLE 1.

Life-history characteristics predicted to be associated with the different strategies of sea urchins (modified from Lawrence 1990).

Strategy	Competitive	Stress-Tolerant	Ruderal
Life-history			
Growth rate	High	Low	Very high
Longevity	Long	Very long	Short
Time to maturity	Short	Long	Very short
Structure			
Strength of test and spines	Relatively strong	Very strong	Weak
Reproduction			
Reproductive effort	High	Low	Very high
Phenology of reproduction	After period of maximum feeding	No general relation to feeding	Early in life
Frequency of reproduction	Usually every year	Intermittent	Every year
Physiology			
Effect of low food level on growth	Great	Small	Very great
Resistance to starvation	Moderate	High	Low
Rate of respiration	High	Low	Very high
Capacity to feed	High	Low	Very high
Resistance to environmental stress, including pollutants	High	Very high	Low
Secondary metabolites			
Palatability	Various	Low	Very high
Phenotypic plasticity	High	Low	Moderate

the same conditions. Small *Echinometra mathaei*, *Diadema setosum*, and *Tripneustes gratilla* fed algal turf in the same aquaria show much different growth (Table 2). The size at age 1 year also indicates species-specific variability in growth rate (Table 3). The growth of small individuals is important, because it is associated with time to sexual maturity (Table 4).

Although these studies are indicative of differences in growth among species, they provide no information on production in energy units. Thus, measurement of growth in species with massive body walls containing large amounts of calcium carbonate is inflated in terms of production, and an increase in volume can involve an increase in mass related to an increase in coelomic fluid and not from production. The data do indicate, however, considerable differences that would be expected with different life-history strategies. The toxopneustids (e.g., *Tripneustes*, *Lytechinus*) grow faster and reach sexual maturity sooner than the echinids (e.g., *Paracentrotus*, *Loxechinus*) and stronglycentrotids (e.g., *Strongylocentrotus*).

Longevity

Ebert (1975, 1982) pointed out the difference in longevity of sea urchin species and its general negative correlation with growth rates. His survey (1975) showed greater longevity of *Echinus esculentus*, *Strongylocentrotus purpuratus*, and *Strongylocentrotus franciscanus* than those of *Lytechinus anamesus*, *Diadema antillarum*, and *Tripneustes ventricosus*. His own studies (1982) showed that *Tripneustes gratilla* is essentially an annual species, with a longevity of about 1 year, and species such as *Heterocentrotus mammillatus* and *Heliocidaris erythrogramma* have a probability of annual survival of over 0.9. Reported longevity (Table 5) certainly vary among the species. Even these higher longevity for the Strongylocentrotidae may be underestimates, given that Ebert (1988) calculated a longevity for *S. franciscanus* of 100 years or more. Russell et al. (1998) calculated a similar longevity for *Strongylocentrotus droebachiensis* in Maine and concluded it also is slow growing and long-lived. This inverse relation between growth rate and longevity should be indicative of the relative allocation of resources to production of biomass and to maintenance.

Reproduction

Gonadal production usually has been measured as the reproductive output (difference between the maximum and minimum gonad index) and not reproductive effort (relative amount of energy consumed or absorbed that is used for gonad production) (Lawrence 1985, 1987). Although the amount of gonad production is important, the relative allocation to gonad production is necessary to evaluate life-history strategies. The reproductive effort, thus, is useful for aquaculture. A species that allocates most of the

TABLE 2.

Growth (mean mg wet body weight \pm SD) of *Echinometra mathaei*, *Diadema setosum*, and *Tripneustes gratilla* in aquaria at Eilat, Israel (Lawrence, unpub.).

Species	<i>Echinometra mathaei</i>	<i>Diadema setosum</i>	<i>Tripneustes gratilla</i>
23 Oct. 1969	118 \pm 12	142 \pm 23	246 \pm 92
22 April 1970	1,175 \pm 92	4,235 \pm 364	25,454 \pm 3,207
Percentage increase	9.9	29.7	103.4

TABLE 3.

Size (mm horizontal diameter) of sea urchin species at an age of 1-year.

Species	Size	Reference
Order Diadematoidea		
Family Diadematae		
<i>Diadema antillarum</i>	40	Lewis 1966
<i>Diadema setosum</i> ^a	45	Drummond 1993
Order Phymosomatidae		
Family Stomechinidae		
<i>Stomopneustes variolaris</i>	15	Drummond 1993
Order Temnopleuroida		
Family Toxopneustidae		
<i>Lytechinus variegatus</i> ^a	50	Moore et al. 1963
	50	Allain 1975
	50–70	Oliver 1987
<i>Tripneustes gratilla</i> ^a	50	Shokita et al. 1991
	70	Dafni 1992
	40	Maharavo 1993
<i>Tripneustes ventricosus</i> ^a	80–90	Lewis 1958
Order Echinoida		
Family Echinidae		
<i>Echinus esculentus</i> ^a	35	Comely and Ansell 1988
	15	Gage 1992
<i>Loxechinus albus</i> ^a	35	Bustos et al. 1991
	20	Gebauer and Moreno 1995
<i>Paracentrotus lividus</i> ^a	7–15	Cellario and Fenaux 1990
	12	Brias and LeGall, in Leighton 1995
	8–11	Willis, in Leighton 1995
	5–16	Leighton 1995
	8	Jangoux (pers. comm.)
	20	Shpigel (pers. comm.)
Family Echinometridae		
<i>Echinometra mathaei</i>	25	Drummond 1993
Family Strongylocentrotidae		
<i>Strongylocentrotus droebachiensis</i> ^a	10–15	Miller and Mann 1973
	15	Fletcher et al. 1974
	ca. 15–20	Sivertsen and Hopkins 1995
	ca. 5	Meidel and Scheibling 1998
<i>Strongylocentrotus franciscanus</i> ^a	20	Bernard and Miller 1973
	13	Ebert and Russell 1993
<i>Strongylocentrotus intermedius</i> ^a	15	Fuji 1960
	<10	Kawamura 1964
	18	Taki 1986
<i>Strongylocentrotus nudus</i> ^a	30	Fuji 1960
	16	Kawamura 1966
<i>Strongylocentrotus purpuratus</i> ^a	18	Kenner 1992

^a Species of economic interest.

feed consumed to maintenance will have a low assimilation efficiency. Although stress-tolerant species may produce large gonads, they would not be as cost effective as ruderal species. The numerous reports of reproductive output in the literature are important but are not useful for this evaluation of the efficiency of gonad production.

Structure

Ebert (1982) found both the development of the test and spines and hydrodynamic exposure of sea urchin species varied independently (Table 6) and were related to survival. At similar exposures,

TABLE 4.
Age (months) and size (mm horizontal diameter) at sexual maturity of sea urchins.

Species	Age	Size	Reference
Order Diadematoidea			
Family Diadematidae			
<i>Diadema setosum</i>	6		Drummond 1993
Order Phymostomatidae			
Family Stomechinidae			
<i>Stomopneustes variolaris</i>	18–24	27	Drummond 1993 Drummond 1991
Order Arbacioidea			
Family Arbaciidae			
<i>Arbacia punctulata</i>		10–60	Harvey 1956
Order Temnopleuroidea			
Family Toxopneustidae			
<i>Tripneustes gratilla</i> ^a	16	60–70	Shokita et al. 1991
	9	40	Dafni & Tobol 1986/87
	10	50	Maharavo 1993
<i>Tripneustes ventricosus</i> ^a	8	20–30	Lewis 1958
		30–45	McPherson 1965
<i>Lytechinus variegatus</i> ^a	12	40–50	Moore et al. 1963
Order Echinoida			
Family Echinidae			
<i>Echinus esculentus</i> ^a	18–30	94–166	Nichols et al. 1985
<i>Loxechinus albus</i> ^a	24	42	Guisado (pers. comm.)
<i>Paracentrotus lividus</i> ^a		15–20	Jangoux (pers. comm.)
Family Echinometridae			
<i>Evechinus chloroticus</i> ^a	18–30	30–50	Dix 1970
	<50–65		McShane et al. 1996; McShane and Anderson 1997
<i>Echinometra mathaei</i>		12	Drummond 1993
Family Strongylocentrotidae			
<i>Strongylocentrotus droebachiensis</i>	40	29	Sivertsen and Hopkins 1995
<i>Strongylocentrotus franciscanus</i> ^a	24	30	Bernard & Miller 1973
		40	McBride (pers. comm.)
<i>Strongylocentrotus intermedius</i> ^a	24	15–35	Kawamura and Taki 1965; Kawamura 1973
	18	20–30	Fuji 1960; 1967
<i>Strongylocentrotus nudus</i> ^a		15	Fuji 1960
		30–40	Agatsuma 1997
<i>Strongylocentrotus purpuratus</i> ^a	12	25	Gonor 1972
		16	Kenner & Lares 1991

survival was directly related to body wall mass. At similar body wall mass, survival was directly related to exposure. Drummond (1993) also found a direct correlation between body wall mass and the effect of hydrodynamics on these three species. Allocation of resources to structural defense is a characteristic of species with a low potential for production and decreases growth rate, increases maintenance, and decreases reproduction (Lawrence 1990).

Capacity to Feed

Ebert (1975) concluded the available studies did not indicate a variability in feeding rate associated with differences in growth and survival of sea urchin species. This is not expected, because all allocations of energy on the right side of the energy-budget equation depend upon the amount consumed. Traits that increase the capacity to obtain and ingest food of higher quality would be predicted to be greatest in species of the ruderal or competitive strategy. The capacity to obtain drift seaweed decreases or even eliminates the dependence of sea urchins to *in situ* production. *Tetrapyge niger* has a greater feeding rate on encrusting algae,

and *L. albus* has a greater feeding rate on drift algae (Contreras and Castilla 1987). The aboral tube-feet of *L. albus* are abundant and have large suckers, and those of *T. niger* are less abundant and have a minimal sucker. *Tetrapyge niger* has a larger Aristotle's lantern than *L. albus*, associated with its grazing feeding behavior. On the Mediterranean coast, *Paracentrotus lividus* feeds on erect and drift algae, and *Arbacia lixula* feeds on encrusting algae and algal tufts (Frantzis et al. 1988). The feeding rate of *Lytechinus variegatus* is greater than that of *Arbacia punctulata* (Hill 1998). It is probable this difference found between other grazing and browsing species is responsible for a greater productivity in *P. lividus*, *Tripneustes* spp., and *Strongylocentrotus* spp.

Feeding rates are affected by temperature, size, physiological and reproductive state, and even the physiognomy of the food (Klinger 1982). Thus, studies of species under the same conditions as done by Contreras and Castilla (1987) provide best comparative information.

Phenotypic plasticity of the Aristotle's lantern, an inverse relation between the availability of food and the relative size of the Aristotle's lantern, has been reported for *Paracentrotus lividus*

TABLE 5.

Longevity (years) of sea urchin species.

Species	Longevity	Reference
Order Diadematoidea		
Family Diadematidae		
<i>Diadema setosum</i>	3–5	Drummond 1993
Order Phymosomatoida		
Family Stomechinidae		
<i>Stomopneustes variolaris</i>	15–20	Drummond 1993
Order Temnopleuridae		
Family Toxopneustidae		
<i>Lytechinus variegatus</i> ^a	2	Moore et al. 1963
	2–3	Allain 1975
<i>Tripneustes gratilla</i> ^a	ca. 1	Ebert 1982
	1 to several	Dafni and Tobol 1986/87
	8	Maharavo 1993
Order Echinoida		
Family Echinidae		
<i>Echinus esculentus</i> ^a	12	Nichols et al. 1985
	>9	Comely and Ansell 1988
	6–10	Gage 1992
<i>Paracentrotus lividus</i> ^a	>10	Allain 1978
	>14	Delmas 1992
Family Echinometridae		
<i>Evechinus chloroticus</i> ^a	>15	Dix 1972
<i>Echinometra mathaei</i>	8–10	Drummond 1993
Family Strongylocentrotidae		
<i>Strongylocentrotus droebachiensis</i> ^a	ca. 8	Propp 1977
	ca. 10–12	Sivertsen and Hopkins 1995
	>50	Russell et al. 1998
<i>Strongylocentrotus franciscanus</i> ^a	18	Breen and Adkins 1976
	ca. 12	Ebert and Russell 1993
	>100	Ebert 1998
<i>Strongylocentrotus intermedius</i> ^a	7–10	Taki 1986
<i>Strongylocentrotus nudus</i> ^a	>10	Agatsuma 1997

^a Species of economic interest.

(Régis 1978), *Diadema setosum* (Ebert 1980), *Echinometra mathaei* (Black et al. 1982, 1984), *Strongylocentrotus purpuratus* (Ebert 1982), and *Diadema antillarum* (Levitan 1991). However, Ebert and Russell (1993) found two populations of *Strongylocentrotus franciscanus* showed no similar phenotypic plasticity of the Aristotle's lantern, nor did Lawrence et al. (1996) for *Tetrapygus niger* or Lawrence et al. (1998) for *Strongylocentrotus droebachiensis*. Ebert and Russell (1993) suggested a possible basis for the lack of the anticipated plasticity of the Aristotle's lantern in *S. franciscanus* was a tighter control of development than in the other species. Lawrence et al. (1996) suggested the lack of phenotypic plasticity of the Aristotle's lantern of *T. niger* would be predicted for stress-tolerant species and could involve tighter control of size.

Respiration and Metabolism

Consideration of respiration and metabolism is complex, because they are involved both in production (anabolism) and in maintenance (repair). We would expect relative allocation to

TABLE 6.

Intercept (α) of the allometric relationship between the body wall wet mass and the total wet body mass of sea urchin species.

Species	Intercept	Exposure rank ^a	Reference
<i>Heterocentrotus mamillatus</i>	0.919	4	Ebert
<i>Colobocentrotus atratus</i>	0.788	1	Ebert
<i>Stomopneustes variolaris</i>	0.718	—	Drummond
<i>Echinometra mathaei</i>	0.672	3	Ebert
	0.607	—	Drummond
<i>Diadema savignyi</i>	0.516	—	Drummond
<i>Echinothrix diadema</i>	0.570	4	Ebert
<i>Strongylocentrotus purpuratus</i> ^b	0.559	7	Ebert
<i>Heliocidaris erythrogramma</i> ^b	0.537	5	Ebert
<i>Strongylocentrotus franciscanus</i> ^b	0.530	7	Ebert
<i>Lytechinus anamesus</i> ^b	0.502	8	Ebert
<i>Tripneustes gratilla</i> ^b	0.398	4	Ebert

^a 1: most exposed; 11: least exposed.^b Species of economic interest.

Modified from Ebert 1982 and Drummond 1993.

anabolism to be greatest in ruderal species and maintenance to be greatest in stress-tolerant species. Energy budgets have not distinguished these uses. As with growth, calculation of energy associated with respiration in terms of wet or dry mass can be misleading, because they do not indicate metabolically active tissue. Use of total organic matter or total protein does not completely resolve the difficulty, because they both include metabolically inactive organic compounds. Rates calculated on a cellular basis (i.e., DNA concentration) are necessary.

Activity of metabolic enzymes should be indicative of innate metabolic rate. The specific activities of glycolytic and hexose-monophosphate-shunt enzymes were usually highest in *Lytechinus variegatus*, intermediate in *Echinometra lacunter*, and lowest in *Arbacia lixula* (Bianconcini et al. 1985), as would be predicted from other characteristics of these species.

A high resistance to starvation can be considered an indication of low basal metabolism. *Eucidaris tribuloides*, *Echinometra lacunter*, and *Lytechinus variegatus* were predicted *a priori* to be resistant to starvation in that order. Individuals starved in the same aquarium with recirculating seawater met the prediction (Fig. 1). Similarly, the loss in biomass with starvation is much greater for *Lytechinus variegatus* than for *Arbacia punctulata* (Hill 1998).

Palatability

The eggs of broadcast spawning sea urchins are vulnerable to predation. A means of preventing predation is to decrease palatability by producing secondary metabolites. However, simply being unpalatable is not sufficient unless increased fitness results. Fisher (1958) proposed that if only one or a few siblings from a large brood are sampled, and the predator learns to avoid others, the frequency of the gene for unpalatability should increase through kin selection. It is necessary that the predator can recognize the unpalatable food. Just as unpalatable insects are often brightly colored, it is possible the unpalatability of arbacioid eggs results from the purple echinochrome, a polyhydroxynaphthoquinone, they contain (McClendon 1912). The concentration is

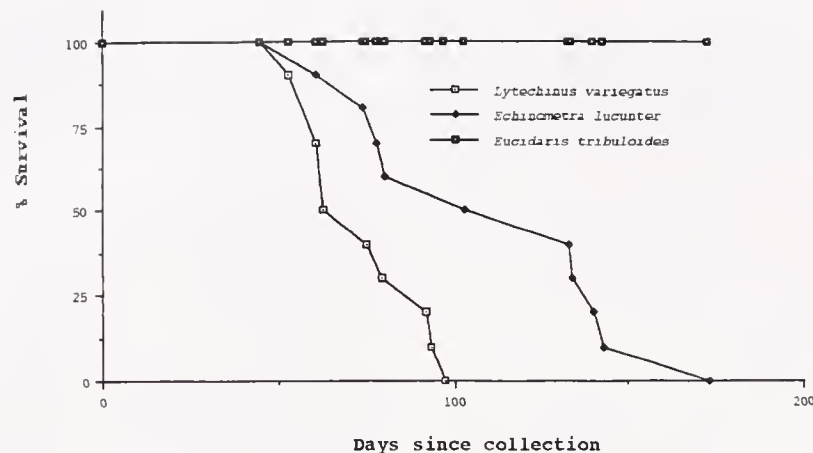


Figure 1. Time to death (days) from starvation of *Lytechinus variegatus*, *Echinometra mathaei*, and *Eucidaris tribuloides* (Lawrence and Lares, unpub.).

0.58 g/100 ml of eggs of *Arbacia punctulata* (Ball and Cooper 1949). Such a response can be interpreted to be indicative of stress-tolerant species in which fecundity is limited and a mechanism to increase the probability of survival of offspring, even if costly. Palatable eggs without secondary metabolites would be expected in species with a greater capacity for production, the more ruderal and competitive species, such as toxopneustids and stronglycentrotids, which are the species of interest for aquaculture.

Conclusions

A consideration of sea urchin species suggest they have a suite of coordinated characteristics that may be associated with life-history strategies. These strategies can be interpreted in terms of energy budgets, which indicate the relative allocation of resources to various functions. The data certainly are not conclusive; in many cases, they simply are not available. We should not expect that all characteristics will indicate unambiguously a particular strategy. Not only can characteristics interact in complex ways, but species may have secondary strategies associated with environments that differ in degree of stress and disturbance that result in intermediate levels of the characteristics. Phenotypic plasticity can occur and

complicate interpretation, particularly in the species with the competitive strategy.

Nevertheless, a perusal of the characteristics indicates a broad difference in strategies. More toward the ruderal strategy are the toxopneustids. More toward the stress-tolerant strategy are the cidaroids and some echinometrids. More toward the competitive strategy are the echinids and stronglycentrotids. The toxopneustids would be most appropriate for aquaculture, because they seem to allocate more energy to production than to protection and maintenance. As a consequence, they grow rapidly and have great roe production at an early age. The echinids and stronglycentrotids allocate relatively less to production. They would grow less rapidly and have less reproductive effort.

Knowledge of the basic biology of the sea urchins that have evolved to increase the fitness of the species in particular environments is essential to evaluating the potential of sea urchins for aquaculture and for establishing their aquaculture. It is essential that energy budgets be prepared for the species considered for aquaculture. This is not a small undertaking, because it will require consideration of size, reproductive state, temperature, and feeding regime. This basic information, which will be directly applicable to aquaculture of sea urchins, is lacking for most species.

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LAND-BASED, CLOSED-CYCLE ECHINICULTURE OF *PARACENTROTUS LIVIDUS* (LAMARCK) (ECHINOIDEA: ECHINODERMATA): A LONG-TERM EXPERIMENT AT A PILOT SCALE

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ABSTRACT Today, most sea urchins fisheries worldwide must deal with overexploitation. Better management of exploited field populations and/or aquaculture is increasingly considered necessary to sustain sea urchin production in the future. In this context, we evaluate the potential of land-based, closed-cycle echiniculture. A long-term experiment with the edible sea urchin *Paracentrotus lividus* has been done on a pilot scale. The process allows total independence from natural resources, because the entire biological cycle of the echinoids is under control (closed-cycle echiniculture), and all activities are performed on land. Furthermore, a method has been set up to control the reproductive cycle with the aim to produce marketable individuals all year long. Performances obtained on each stage of the rearing process are quantified and analyzed. Overall, the results of this experiment are promising; however, some problems remain to be solved before we can claim profitability. The most important finding is that land-based, closed-cycle echiniculture is a potential viable supplement to fisheries to sustain worldwide sea urchin roe production.

KEY WORDS: Sea urchin, *Paracentrotus lividus*, aquaculture, larval culture, metamorphosis, growth, roe enhancement

INTRODUCTION

Depending upon their respective gastronomic cultures, people consider sea urchin gonads (both male and female gonads are collectively referred to as roe) as either a fine and delicate seafood or as absolutely inedible. However, its economic value is well established given the price consumers are willing to pay. The wholesale price of live sea urchins in France ranges from 30 to 120 FF/kg (price range in the 1990s at Rungis, Paris) and fresh roe in Japan from 6,000 to 14,000 ¥/kg (price in Japan in the 1990s, see Hagen 1996). Both market prices are roughly equivalent in terms of fresh roe, making sea urchin roe one of the most valuable seafoods in the world. In both markets, the lowest prices are those of imported sea urchins, which are considered to be of poorer quality.

The most important market, Japan, imports approximately five thousand tons of sea urchin gonads per year, the equivalent of 40 to 50 thousand tons of live sea urchins (Hagen 1996). According to the same author, the Japanese consume approximately 60,000 tons of whole sea urchins per year. The second largest consumer is France, with an annual consumption of approximately 1,000 tons of whole sea urchins (Le Gall 1990).

Increasing demand for sea urchin roe and a steady rise in price have led to worldwide intensification of sea urchin fisheries (Conand and Sloan 1989, Le Gall 1990, Saito 1992), which has now (1998) probably reached its maximum. This production cannot be sustained at current levels, because the declining productivity of overexploited existing stocks can no longer be compensated by harvest of new stocks, as was possible over the last three decades (most exploitable natural populations have already been fished). In Japan, this decline occurred despite the development and imple-

mentation of extensive domestic fishery enhancement techniques, which include the annual release of 60 million juvenile sea urchins into the wild (Saito 1992, Hagen 1996). Consequently, the worldwide supply of high quality sea urchin roe will be unable to meet market demand unless commercial sea urchin aquaculture develops to partially replace the steady decrease in natural captures.

Aquaculture of echinoderms, including sea urchins and sea cucumbers, is known as echinoculture (Le Gall and Bucaille 1989, Le Gall 1990, Hagen 1996). We prefer to use the term echiniculture to describe sea urchin aquaculture exclusively (Echinoidea); thus, it is more accurate in this context. This activity is not yet fully developed. Maintenance or rearing of sea urchins in the laboratory has been successfully performed for different species (Hinegardner 1969, Fridberger et al. 1979, Cellario and Fenaux 1990). Several different processes are being experimented on a larger scale, ranging from sea urchin ranching (cultivation in the field, see Fernandez and Caltagirone 1994, Fernandez 1996), to land-based systems (Le Gall and Bucaille 1989, Le Gall 1990, Fernandez 1996) or polyculture (sea urchins cultivated in cages with fish, see Kelly et al. 1998). Nevertheless, considering the limited carrying capacity of natural sites that are already largely exploited by fisheries, only land-based or cage techniques will help to sustain worldwide sea urchin roe production. Similarly, only cultivation processes totally independent of natural stocks; that is, by controlling the complete life cycle of the echinoid, will lower the pressure imposed by fisheries upon natural populations. In this context, this paper presents a 7-year experimental rearing method to produce the edible sea urchin *P. lividus* on a pilot scale, and discusses the biological and technological issues that emerged from this cultivation method.

MATERIAL AND METHODS

The aim of land-based, closed-cycle echiniculture is to get maximum control over each phase of the sea urchin's life cycle by controlling major environmental parameters (temperature, photoperiod, water quality, quality and quantity of food). A land-based system has advantages over rearing methods performed directly in the sea. The greatest of these is the ability to control the whole life cycle of the animal (closed cycle), thus the sea urchin never depends, at any stage, on a supply of animals originating from the field.

The method used here is adapted from Le Gall (Le Gall and Bucaille 1989, Le Gall 1990) with some fine-tuning and modifications that allow a routine output of sea urchins on a pilot scale. An experimental facility was set up at the Centre de Recherche et d'Etude Côtière (CREC, Normandy, France) in which several generations of sea urchins were reared according to a thoroughly defined experimental procedure.

Pilot Echiniculture Facility

The experimental facility includes a hatchery (30 m³) and a cultivation room (160 m³). The hatchery is equipped with 11 200-L larval rearing tanks (see below) and a system for phytoplankton production (classical devices for large-scale production).

The cultivation room is insulated, thermoregulated at 22°C ± 1°C, correctly aerated, and exposed to a 12h/12h photoperiod. It is equipped with 10 autonomous rearing structures with either three or six superposed 4-m long and 60-cm wide ponds called toboggans. Each set of toboggans hangs over a reserve/settling tank of the same length, 80 cm wide and 80 cm deep. The water depth in the toboggans varies between 5 and 10 cm. A centrifugal pump transfers water from the reserve tank to the top level, with a flow of 8 to 10 m³/h (4 to 5 m³/h for the pregrowth structure, see below). The water then recirculates by gravity from one level to the other (each toboggan has a gentle slope to help water run into it and is connected to the previous and the next one at its opposite ends, see Fig. 1). This device, specifically designed for sea urchin cultivation, optimizes both the surface available for the postmetamorphic individuals and the water current around them. It also facilitates access to the animals and their visual control. The 10 rearing structures are organized as follows:

- (1) One pregrowth structure of 3 toboggans with a capacity of 1,500 L of circulating water thermoregulated at 20°C ± 1°C. The water is renewed at a rate of 150% per day. This structure can hold a biomass between 0.2 and 1 kg/m² of toboggans.
- (2) Two growth structures made of six toboggans each. The capacity of each structure is 3,000 L of circulating water thermoregulated at 18°C ± 1°C and with a water renewal ranged between 100 and 600% per day, depending upon the density of sea urchins present in the structures. These structures can hold a maximum biomass of 7 kg of sea urchins per m² without supplemental water filtration.
- (3) Seven experimental/conditioning structures of three toboggans each with a capacity of 1,500 L of circulating water. These are isolated from one another so they can be thermoregulated individually from 10°C to 25°C, and each has up to six different photoperiods (a dark separation divides the toboggans in their center). An electronic system allows the transition of light to darkness and vice versa, thus simulta-

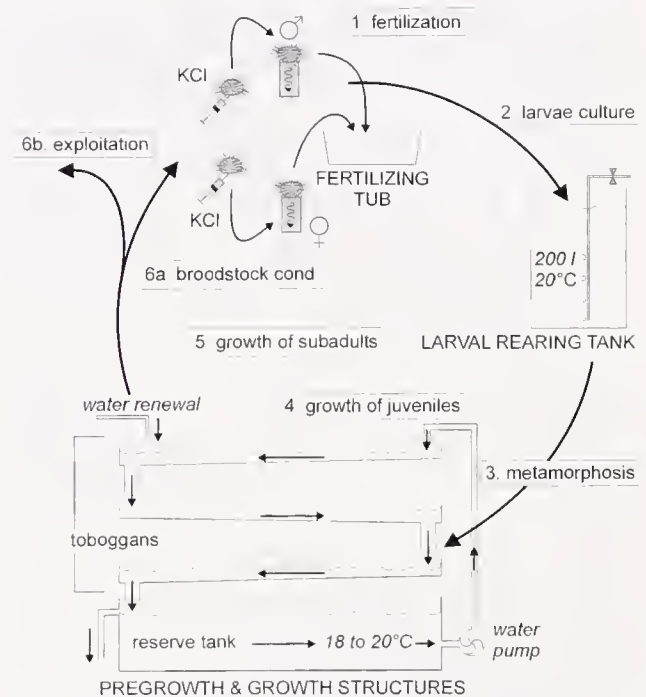


Figure 1. Overview of the closed-cycle process and devices used to produce sea urchins on land at a pilot scale.

lating dawn and dusk. The rate of water renewal can be fixed between 50 and 600% per day. Biomass varies following criteria imposed by the experiments.

Additional devices are grouped in a technical room containing a central thermoregulation system (a thermorefrigerating pump providing either cold or hot water to the heat exchangers that equip the rearing structures), a water pumping and filtration system, an emergency electric generator, and a central alarm. The water is pumped directly from the sea at high tide and is stored in a reservoir of 60 m³. It is filtered before being used (30-µm mesh cartridge mechanical filtration, followed by a 14 m³ biological filter and two settling tanks of 8 m³ each).

Origin of the Animals

The species cultivated is *Paracentrotus lividus* Lamarck (1816). This species is found all along the European coast from the northern Atlantic Irish coast to the Mediterranean Sea. All individuals used come from a single population located in Morgat, Brittany, France. Some were directly collected in the small tide pools that spread all along the rocky shores of Douarnenez Bay (emerged only during high coefficient tides). The remaining animals come from artificial fertilizations in the laboratory and were grown in the structures [cross fertilizations of first (F1) or second (F2) generation of sea urchins collected in the field]. By so doing, the age and the parental origin of the F1 and F2 sea urchins are known precisely.

Rearing Method

Aiming at closely matching the echinoid requirements along their life history and minimizing technical constraints, the dissociation of the whole rearing cycle into seven stages is essential (Fig. 1). These stages are: (1) fertilization; (2) larval culture; (3) metamorphosis; (4) growth of juveniles; (5) growth of subadults;

and (6) growth of adults, which is further divided into (6a) conditioning for marketing of roe (exploitation); and (6b) providing gametes (broodstock).

Stage 1

Fertilization is performed using gametes issued from healthy animals that restored their gamete potential as described below in broodstock conditioning (stage 6b). The gametes are obtained by stimulating the parents to spawn with 0.5 N KCl (injection of 50 μ L per g of body weight through the peristomial membrane). The gametes of each individual are collected in a small jar of 50 mL in 20°C filtered natural seawater (on a 1 μ m cartridge filter, referred to hereafter as "larval rearing water").

When the spawning is over, the volume of the gametes is evaluated. The ova of a single female are transferred in a fertilization tub; that is, a shallow polyethylene container. The volume is brought to 800 mL with the same water. One fifth of the spermatozoa of a single male is added to the ova. The mixture is kept at $20 \pm 1^\circ\text{C}$ during 4 h, and the tub is gently stirred three or four times during that period. After that, the success of the fertilization is checked, and the fertilized eggs are counted (most often over 90% of the eggs are fertilized).

Stage 2

Rearing of the larvae is done in a 200-L polyethylene cylindrical tank where larval rearing water is introduced 24 h beforehand and stabilized at $20 \pm 1^\circ\text{C}$. The embryos (in the gastrula stage) are introduced at a concentration of 250 per liter. This density is low enough to allow the entire rearing of the larvae to be conducted without renewing the water. The food (*Phaeodactylum tricornutum* Bohlin issued from cultivation in Erdschreiber medium) is introduced from the third day postfertilization (acquisition of larval exotrophy). The larvae are fed once a day with 600 mL of bloom algal cultivation (concentration around 10×10^6 cells/mL). The whole is kept in dim light with a 12h/12h photoperiod and is gently mixed and aerated by central bubbling.

Stage 3

From the sixteenth day onward, competence to *metamorphosis* is checked daily (Standard Competence Test or SCT, adapted from Gosselin and Jangoux 1996). One hundred larvae are transferred in a clean SCT sieve (a 10-cm high, 20 cm² sieve with a bottom mesh of 250 μ m placed 1.5 cm above the water floor). This SCT sieve is placed in the pregrowth structure in the presence of a metamorphosis stimulating factor (living *Corallina elongata*, Ellis and Solander, freshly collected from the field). The percentage of metamorphosed larvae is determined 24 h later. If this value lies around 80%, the whole batch is transferred in the pregrowth structure aiming at its fixation on one or two metamorphosis sieves (similar to SCT sieves but each covering 1,800 cm²). Batches containing large amounts of larvae exhibiting bad development, abnormalities, or too low metamorphosis ratios are discarded.

Stage 4

Growth of juveniles. The postmetamorphic period begins with a short endotrophic stage. During this period, the postmetamorphic individuals, also called postlarvae, reorganize their digestive tract (Gosselin and Jangoux 1998). The mouth and anus of the future juvenile are not yet pierced. This postlarval stage lasts for up to 8 days, after which the echinoids become exotrophic juveniles. One

or two days before development of exotrophy, 100 g fresh weight of *Enteromorpha linza* (L.) Agardh collected in the field are distributed in each sieve. From this moment onward, the same food quantity is given every time it is completely consumed. Some *Gammarus locusta* L. are also introduced to clean the sieves of decomposing parts of the algae.

The juveniles are left in these sieves until the mean individual size in the batch reaches 2 mm. The entire batch is then transferred in 500 μ m mesh pregrowth sieves. A homogeneous bed of *E. linza* is maintained in the sieves. The bottoms of the sieves are cleaned every week using filtered seawater. Because the growth of the juveniles is not homogeneous (Grosjean et al. 1996), the animals are graded each month, and those with a diameter greater than 5 mm are transferred into a 1-mm mesh pregrowth sieve. The *E. linza* diet is maintained, and the sieves remain in the same pregrowth structure.

Stage 5

Growth of subadults. Each month, sorting of size is done to collect all individuals over 10 mm. The individuals whose size exceeds 10 mm but is below the minimum market size of around 40 mm for *P. lividus* are defined as subadults. They are potentially mature but not large enough for market. Consequently, their somatic growth performances must be promoted, while their gonadal growth should be kept as low as possible to optimize food allocation to the soma.

Subadults are placed in rectangular rearing baskets, with all sides made of 5-mm mesh. These rearing baskets are placed 1.5 cm above the bottom of the toboggans and are just slightly narrower. This is important to allow good water circulation around and inside them, and good elimination of solid wastes produced by the sea urchins. Their surface ranges between 1,200 and 2,400 cm². When the size of the animals increases above 15 mm in test diameter, they are transferred in the same type of rearing baskets, but with 10-mm mesh, which allows even better water circulation.

Subadults, inside their rearing baskets, are transferred to a growth structure. From this time onward, and twice a week, they are fed *ad libitum* with fresh kelp, *Laminaria digitata* (Hudson) Lamouroux. Cleaning of the baskets and toboggans is also done twice weekly. Dead or dying animals are removed daily. Each month, sorting by size is done to separate the batches into different size categories from 5 to 5 mm. The entire cultivation is kept in 12h/12h photoperiod.

Stage 6a

Conditioning adults for market. When the sea urchins reach 40 mm, they are prepared to get marketable gonads in conditioning structures. For commercialization, it is of the utmost importance that the echinoids' gonadal cycle is synchronous, presents the right stage of maturity (reproductive stages 4 and 5, growing and premature, according to Spirlet et al. 1998a), and is of acceptable texture (firm and not leaking), size (as large as possible), good color (yellow-orange to bright orange), and taste. *P. lividus* has an annual reproductive cycle that tends to fade in constant artificial conditions; lacking the "usual" stressors (low temperature, lighting variation, lower quality or lack of food during winter), the echinoids tend to bypass the growth phase of the gonads and have permanent gametogenesis, giving rise to flabby gonads with few nutritive phagocytes. Such gonads are unacceptable in the market. To counteract this, the echinoids are starved at a temperature of 12

to 14°C and at a 12h/12h photoperiod. This leads to consumption of the possible content of the gonads, which also act as storage organs, in order for the animals to get in phase regarding their reproductive cycle (reproductive stages 1 to 3, spent and recovering, Spirlet et al. 1998a). When the content of the gonads is fully consumed; that is, between 1 and 2 months later, depending on their initial state, sea urchins are fed *ad libitum* with either *Laminaria digitata* or an appropriate artificial food rich in proteins (Klinger et al. 1994, Klinger et al. 1997, Klinger et al. 1998, Williams and Harris 1998) at a higher temperature (at least 16°C). The duration of this feeding stage is dictated by the maturation of the gonads and lasts for 6 weeks to 3 months, mainly depending on the food quality. Usually, both the size and the maturation stage simultaneously reach acceptable values, and gonads are ready for the market at the end of this starving-feeding treatment (see results).

Stage 6b

Conditioning broodstock. Maintaining mature broodstock of *P. lividus* all year long is done by keeping individuals at high temperature (between 18°C and 20°C) and under either a fixed photoperiod of 12h/12h (directly in the growth structures) or, better, in total darkness (in a conditioning structure), leading to the disruption of their reproductive cycle. In these conditions, food is the most important factor to get large quantities of good quality gametes. Feeding adults *ad libitum* with fresh *Laminaria digitata* ensures both the quality and the quantity of sexual output. The quality of gametes is often a little bit lower from December till February, although still usable most of the time.

Measurements of Reared Sea Urchins

Essentially two criteria are used to quantify the performances of the rearing method: (1) the survival rate with time; and (2) the growth rate; that is, the change of test diameter of the urchins with time (gonadal size and quality are taken into account only after the minimal market size has been reached). The first is determined by counting survivals in a single batch (issued from a single fertilization and a single larval rearing tank) at various times. The counting of eggs, embryos, and larvae is performed on at least five samples of the homogenized batch (the volume chosen to count each time is at least one hundred individuals), and the total amount is estimated by extrapolating the mean concentration found to the whole volume. The survival rate of competent larvae, postlarvae, and juveniles is determined by rearing subsamples of 50 to 100 individuals in SCT sieves. Several replicates (at least five) are sacrificed and counted at each time. All subadults and adults of a batch are counted and measured every 3 months (typically between a few hundred to a few thousand individuals in each batch) during size sorting. Measurements of subadults and adults do not induce additional stress or mortality other than those occurring during the normal size grading operation (no additional manipulations). Mortality caused by manipulations could thus be attributed to the rearing method itself.

Size is evaluated by means of the diameter, which is measured to the ambitus of the test (its largest part) considered without spines. To prevent errors caused by a possible slightly oval shape, we measure two perpendicular diameters, both to the ambitus, and only the average is considered. The diameter of juveniles, after fixing them (glutaraldehyde 3%), is measured on digitized microphotographs using a specific image analysis software (Grosjean et

al. 1996). The diameter of subadults and adults is measured with a sliding caliper. Fresh weight, used to evaluate biomass, is measured after draining residual water on absorbent paper for 5 minutes.

The relative size of the gonads is quantified by means of the fresh and dry weight gonadal indices (GI, also called gonadosomatic indices). These indices are defined as the ratio between the fresh (or dry) weight of the gonads and the total fresh (or dry) weight of the urchins. First, fresh weight of the urchins is determined after drying them for 5 minutes on absorbent paper. The animals are then dissected, and the five gonads are extracted and weighed. One gonad is fixed in Bonin's fluid for further determination of its gametogenic stage (see below). The remaining four gonads are weighed again, and the difference is computed to allow correction of the dry weight for the missing gonad. The remaining gonads and the soma are then dried at 70°C during 48 h (constant weight) before being separately weighed. Dry weight gonad index is more accurate but has been found to be less representative of the "filling" of the sea urchins (how much space the gonads occupy inside the coelomic cavity), especially when comparing various maturity stages and/or various diets (unpublished results). Both indices are provided to allow comparisons.

The maturity stage is determined on histological sections of the fixed gonad following an 8-stage scale defined by Spirlet et al. (1998a). The maturity index (MI) corresponds to the arithmetic mean of all the observed maturity stages. Male and female data are pooled for both the GI and the MI, because differences between sexes are not significant (Spirlet et al. 1998a, Spirlet et al. 1998b).

RESULTS

Table 1 shows the age, density, and survival rate for each stage described in rearing conditions. These data come from 29 fertilizations studied over several years taking into account, among other things, seasonal variations. The survival rate for larvae is about 56%. Competence is reached most often in 18 days (mode and median value), with an average value of 19.5 days, a minimal time of 16 days and a maximal time of 25 days. The mean metamorphosis rate is 80.4% when larvae are competent. This rate was reached in almost two-thirds of the fertilizations that attained the competent stage (nonsymmetrical distribution). Thirty percent of the larvae were discarded, either because of incomplete development or too low a metamorphosis rate. The remaining larvae were used for studies on postlarval or juvenile stages (and, thus, sacrificed whenever measured) or were reared to the adult stage. Overall, the survival rate is homogeneous from one fertilization to the other and for all stages, except during and after the acquisition of exotrophy (transition from the postlarval to the juvenile stage): the average rate is 54.5%, but extremes are close to 0 and 100% (13% and 94.5%, respectively). Whatever the success of this transition, the most critical period for survival is the juvenile stage, with a very low survival rate of 5%. Most mortality occurs during the few first months of the juvenile's life (and even probably during the few first weeks), with a progressive decrease around 8 to 9 months of age.

Figure 2 shows both the survival rate and the size distribution over time of a batch followed for 7 years, far beyond the minimal marketable size and age. For the sake of clarity, only data taken every 6 months are presented, although measurements were made every 3 months beginning at 6 months of age. The trends observed on this single cohort are representative of the way animals grow in

TABLE 1.
Age, density, number, and survival rate of sea urchins at each rearing stage.

Rearing Stage	Developmental Stage	No. Replicated Fertilizations	Age (Min/Median/Max)	Mean Density (No. Ind./Vol. or/Surf. Unit)	Mean No. Individuals in 1 Batch	Survival from Previous Stage (%) Mean \pm SD	Mean Global Survival Rate (%)
1	Embryos	29 ^a	4 h	250/1	50,000	—	100
2	Competent larvae	29 ^a	16 day/18 day/25 day	141/1	28,200	56.4 \pm 11.6	56.4
3	Postlarvae	18 ^b	idem + 1 day	6.5 \times 10 ⁴ /m ²	22,700	80.4 \pm 14.4	45.3
4	Juveniles	9 ^b	idem + 10 day	3.5 \times 10 ⁴ /m ²	12,400	54.5 \pm 26.8	24.7
5	Subadults	6 ^c	ca. 9 months	4,000/m ^{2d}	600	4.9 \pm 1.5	1.2
6a&b	Adults	5 ^c	1.7 y/2.6 y/3.5 y	250/m ^{2d}	310	51.5 \pm 3.0	0.6

^a Total number of larval rearing tanks: 103, from which 72 have produced enough usable competent larvae.

^b In the pregrowth structure. 5 to 15 replicates are measured at key times for each fertilization (see Material and Methods).

^c In the growth or conditioning structures. Each batch is issued from a single larval rearing tank and is followed over 2 to 7 years.

^d Densities in the rearing structures are adjusted during sorting operations according to both the individual size and the survival rate.

cultivation, as confirmed by the five other independent batches measured over 2 to 4 years (for an illustrated example of another batch, see Grosjean et al. 1996).

Mortality (represented on the backwall of the 3-D box in Fig. 2) remains very high until about 9 months of age in the pregrowth structure. In the figured case, from around 12,400 juveniles issued from one rearing tank only 725 individuals were counted after 6 months and 507 remained after another 3 months. Mortality dropped after this critical period, and 491 individuals were still alive 3 months later (1 year of age). This corresponds, respectively, to a mortality of 94% (between the acquisition of exotrophy by the juvenile to 6 months old), 30% (during the next 3 months), and 3% (after the following 3 months). The mortality rate of subadults stabilizes around 5.4% per trimester until 6 years of age, but ranges from 0.9% per trimester to 12.7% per trimester. Most of this variation is correlated with season: mortality is higher during winter; whereas, summer mortality nearly reaches 0%. Most of winter mortality occurs in waves that start unpredictably and last for 2 to 3 days.

Juvenile's individual growth in test diameter is slow. It accelerates for subadults but then scatters for intermediate sizes (15 to 35 mm), even inside a presumably homogeneous batch. This scat-

tering often results in bimodal or trimodal size distributions (see Fig. 2 for an example and Grosjean et al. 1996 for an analysis). When echinoids approach asymptotic size, their growth rate drops. Hence, the leading group is eventually caught up by the trailers around or slightly above the minimal market size. This minimal market size is attained between 1.7 and 3.5 years old (respectively, 10% and 90% of the individuals are larger than 40 mm), with a median value of 2.6 years.

Biomass variations (Fig. 3, same batch as in Fig. 2) are correlated to both the survival rate and the growth speed of reared echinoids. The higher mortality observed in winter overrides growth speed, and biomass tends to decrease slightly. Summer biomass is highest during the third and the fourth years in this case. The first peak of biomass (around 3.5 years old in the figured case, between 2.8 and 3.5 years old for the other batches, depending on the season) correspond to reaching the minimal market size by more than 90% of the individuals and seems to be the best time to commercialize them after conditioning their gonads (stage 6a) from a strict biological point of view. At that time, between 35 and 40 kg of fresh weight sea urchins are produced in a single batch. This represents an over-all yield per surface unit of the growth structures of 4 to 7 kg/m² of toboggans/year. To obtain this result, roughly 400 kg of kelp was provided to the sea urchins. Thus, over-all food conversion efficiency lies around 10%.

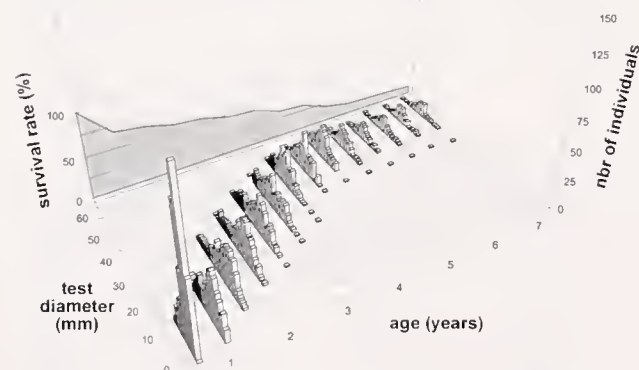


Figure 2. Changes with time in the size distribution and survival rate of one fertilization issued from a single larval rearing tank and followed over 7 years. Note the leading group that singles out (represented by dark bars in the histograms).

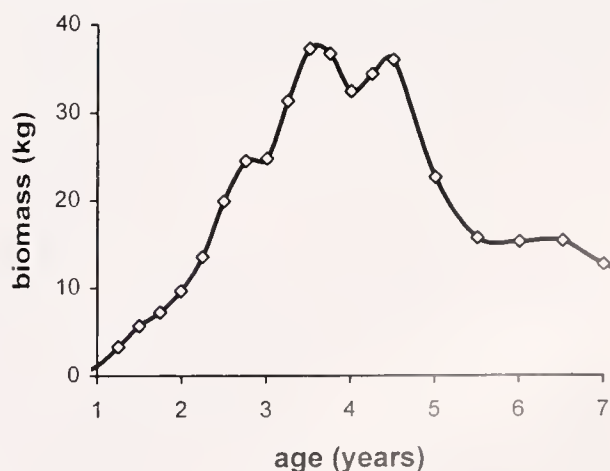


Figure 3. Change with time in the biomass of a reared cohort of sea urchins (the same batch as shown in Fig. 2).

Table 2 presents some results obtained after conditioning the sea urchins for market with the starving–feeding method. To allow comparisons, GI and MI of field echinoids issued from Brittany are also provided. In the field, best GI was observed in March and reached a mean value of 11.6% in fresh weight. Sea urchins conditioned in cultivation show similar GI and MI. The feeding period must be extended to 3 months when using *L. digitata*; whereas, 2 months are sufficient with the artificial diet to obtain the same results. Feeding 3 months with the pellets leads to a remarkable mean GI of 17.5% in fresh weight. Such a GI has never been observed in the field and corresponds to complete filling of the coelomic cavity with the gonads, the digestive tract, almost empty, being compressed against the body wall. However, the MI is too high, and the gonads contain too many gametes to satisfy market criteria. Furthermore, the color obtained with the pellets is too pale (white to beige), and the taste does not match wild roe; whereas, gonads produced with *L. digitata* are of good quality. An out-of-season conditioning was initiated in July with a long-day photoperiod (17h/7h). Very large gonads (GI around 14%) with an adequate MI were obtained in October, after only 6 weeks of feeding with the artificial food. Hence, the starving–feeding method could be used to produce marketable gonads all year long.

DISCUSSION

Both the increasing demand for roe and systematic overexploitation of wild populations support the need for sea urchin cultivation independent of field resources. The method presented here is one design of a rearing process that satisfies this criterion. It seems to be successful at any life stages of *P. lividus* on a pilot scale.

Obtaining gametes of *P. lividus* in large amounts is an easy task, as is the rearing of its larvae with the proposed method (rudimentary devices, low maintenance and feeding with one of the easiest microalgae to grow: *Phaeodactylum tricornutum*). Metamorphosis is a little bit more critical but can be achieved with care and use of a good inductant (fresh coralline algae). Rearing of juveniles, subadults, and adults is feasible if five major constraints are simultaneously respected. A specific design of the rearing structures and baskets provides (1) correct water flow around the echinoids (for gas exchanges and removal of solid wastes) and (2) sufficient bottom surface on which those benthic animals can settle (stacked toboggans). The maintenance of good water quality is ensured by (3) the adaptation of the sea urchin density at each life stage and (4) water renewal fixed at a sufficient rate to minimize pollution and avoid depletion in carbonates (see rearing method for tolerable values for both parameters without supplemental filtra-

tion). Finally, (5) providing adequate food *ad libitum* promotes somatic and gonadal growth. Further regulation of resources allocation is possible by diet (starving–feeding method), temperature, and photoperiod conditions, leading to good quality of the final product—the roe—which could be obtained all year long.

If all these five conditions are met, *P. lividus* behaves fairly well in cultivation and seems highly resistant to diseases. The only cases of disease observed (mainly necrosis on the test or spines) were attributed to opportunistic bacterial or fungal infections attributable to poor rearing conditions; that is, when one or several of these five parameters were poorly controlled. Cannibalism was also observed when the quality of food was low or when carbonates concentration or pH dropped (“foraging” behavior to compensate for the lack in calcium carbonates?) or on dying animals, but never on healthy individuals maintained in good condition.

Growth is perfectly asymptotic, and the maximal size of 45 to 65 mm (individual variation) is reached around 3.5 to 4 years old in the rearing conditions described. This size is similar to that observed among the field population of Morgat, from which reared sea urchins descend directly or indirectly. In Brittany, the most precise estimation of size at age for wild populations of *P. lividus* was performed by Allain (1978) by analysis of the growth bands in the skeleton. According to this author, wild sea urchins reach the size of 40 to 50 mm in 4 years, which is a little longer than in the present rearing conditions (between 2 and 3.5 years). The gain could probably be attributed to the food distributed *ad libitum* all year long and to the water temperature (heating of the water in the winter) as already suggested by Le Gall (1990).

The success of the present method leads to optimistic forecasting for the future of echiniculture. However, we should probably expect slightly different results with large-scale, intensive cultivation. With the experience acquired during this long-term trial and some informal observations performed at a larger scale, we can predict some problems that could potentially arise when scaling up or when considering profit. These problems can be ranged into four different categories: (1) loss of profit because of high and/or uncontrolled mortality of juveniles and subadults; (2) unevenly distributed growth rates caused by intraspecific competition; (3) lack of carbonates and accumulation of CO₂ because of skeletogenesis in intensive closed-circuit systems; and (4) problems linked to the quality of food, water pollution, or poor color and/or taste of gonads produced with artificial diets.

Survival rates around the critical period when the postlarva acquire exotrophy to become fully functional juveniles are highly unpredictable. To get over the difficult phase of endotrophy, the

TABLE 2.
Gonadal and maturity indices of wild and reared sea urchins (pooled results for males and females).

Origin	Month	Food	Treatment	Temperature	Photoperiod (L/D)	Wet W. GI (%) Mean ± SD	Dry W. GI (%) Mean ± SD	MI ^a Mean ± SD
Field	March	Natural diet	Collected in Morgat ^b	10°C	13h/11h	11.6 ± 4.2	7.1 ± 2.6	4.3 ± 0.5
Cultiv.	June	<i>L. digitata</i>	2 mo starving/3 mo feeding	16°C	12h/12h	11.1 ± 2.6	7.3 ± 1.7	4.4 ± 0.5
Cultiv.	May	Pellets ^c	2 mo starving/2 mo feeding	16°C	12h/12h	11.2 ± 3.3	6.7 ± 2.1	4.2 ± 1.3
Cultiv.	June	Pellets ^c	2 mo starving/3 mo feeding	16°C	12h/12h	17.5 ± 2.4	11.3 ± 1.5	6.2 ± 1.0
Cultiv.	October	Pellets ^c	2 mo starving/1.5 mo feeding	16°C	17h/7h	13.9 ± 1.5	9.7 ± 1.0	4.8 ± 0.9

^a Best MI values for the market range from 4 to 5 (growing and premature reproductive stages).

^b Mean values obtained on samplings during 3 consecutive years.

^c For the composition of this food, see Williams and Harris 1998 (Table 1, “new diet”).

larvae must store enough reserves before undergoing metamorphosis. In addition, the early juveniles must promptly find suitable food when their digestive tract becomes functional. It seems that one or both parameters are not always optimal in rearing conditions. In any way, with a mean 55% success rate, we obtain over 12,000 viable juveniles per 200-L tank, which is enough for our use, but can probably be improved. Indeed, gametes are not limited: a female of 40-mm diameter usually produces around 5 to 7 millions of eggs. Thus, the 50,000 embryos introduced in one larval rearing tank represent only about 1% of a whole spawn (about 0.2% of the sperm produced by a single male). Hence, only a few dozen mature adults are necessary to produce enough gametes for mass production of larvae.

However, after the critical phase of exotrophic acquisition, the mortality of juveniles remains very high until they reach about 10 mm in test diameter. To minimize this, juveniles are reared in specific structures referred to as pregrowth structures where biomass is kept at a low level and where water quality is of prime importance. Moreover, quality of the immediate environment of juveniles is improved by use of a good "water-resistant" diet (*Enteromorpha linza*) and by means of cleaners (*Gammarus locusta*). In any case, the space occupied in the pregrowth structure by juveniles and the total care they need remain much lower as compared to subadults and adults (compare densities in Table 1). This minimizes the cost of losing many juveniles from the point of view of the total productivity of the cultivation.

More insidious is the effect of winter mortality of subadults and adults. Its cumulative value is ten times lower than juvenile mortality, but its cost is much higher, because it concerns individuals occupying a significant space in the growth structures and having already consumed a significant amount of food (drop of the overall yield per surface unit and food conversion efficiency). However, the cause of this seasonal variability cannot be explained. It could be because of lower quality of food (fresh kelp with a seasonal variation in their composition, Gayral and Cosson 1973, Abe et al. 1983) or to any pollution of the water probably induced by the food itself (bad quality food is less ingested and decomposes more easily), or to another undetermined cause. For the moment, waves of mass mortality have not been correlated with either temperature variability of the natural seawater, meteorological conditions (atmospheric pressure, rain), or feeding. However, any correlation will be difficult to assess, because of the scarcity of these mass mortality waves and the probable, but not quantified, delay between the stress and the observed mortality. Total productivity could undoubtedly be enhanced if this winter mortality was lowered or eliminated. To suppress or minimize the winter decrease in the biomass is also worth considering when one intends to produce marketable gonads all year long.

Mortality is not the only problem inhibiting steady productivity: widespread distribution of growth speed among individuals expands the time interval when largest and smallest individuals are exploitable and constrains to sort batches frequently. Growth of *P. lividus* is very slow at the juvenile stage. This "lag-phase" has also been observed by Cellario and Fenaux (1990) for the same species in cultivation and by Ebert and Russel (1993) for wild populations of *Strongylocentrotus franciscanus* Stimpson. When growth initiates in term of test diameter, size distribution expands. This individual variability is not genetic but is attributable to a reversible size-based intraspecific competition (Grosjean et al. 1996) that takes place rapidly, even in size-sorted batches, although sorting reduces its effect. Presently, the exact impact of this competition

on productivity and the best way to avoid it (if it should be avoided at all) are still unknown.

A third problem that will probably occur when considering further intensification of echiniculture in closed or semiclosed systems is the depletion of dissolved carbonates and the accumulation of CO_2 in seawater. In growing, the sea urchin builds a magnesium-calcite skeleton. This skeleton represents an important fraction of the body weight: between 28% and 31% of the total fresh weight for *P. lividus* (measured on animals issued from the reared strain, after digestion of organic tissues with a 12%Chl bleaching agent under gentle agitation, $n = 356$). Thus, for each kg of fresh weight produced, about one-third has to be supplied in one or the other form of calcium carbonate. However, *P. lividus* is unable to assimilate efficiently carbonates provided as a solid substrate (calcareous rocks, algae, or cuttlefish bones, for example), because the pH of its digestive tract is too high to dissolve large amounts of solid calcite (between six and eight, for a review see Lawrence 1982, for data concerning *P. lividus* see Claerebout and Jangoux 1985). The main usable source of magnesium/calcium carbonates is thus present under a dissolved form in seawater. If calcium and magnesium ions (respectively 400 mg and 1,350 mg per kg seawater at a salinity of 35‰, Spotte 1991) are not limiting, the quantity of dissolved carbonates available could be consumed very quickly in intensive closed or semiclosed systems (unpublished data). Most of the carbonate alkalinity (about 2.3–2.4 meq/kg seawater, corresponding to 140 mg of HCO_3^-) remains unavailable for skeletogenesis, the pH dropping too much when sea urchins consume it (carbonate and bicarbonate are the most important chemical components that buffer pH in seawater, Stumm and Morgan 1981). The actual fraction the sea urchins can use is still unknown, but is probably under 10% of the total carbonate alkalinity. To illustrate this, without supplemental chemical filtration and with a usable fraction of 10% of the dissolved carbonates to produce skeleton that final weight represents 30% of the total sea urchin fresh weight, one must provide at least 24,500 m^3 of seawater per ton of sea urchin fresh weight produced. However, this optimistic calculation does not consider mortality that otherwise also exports carbonates.

Precipitation of bicarbonates (the main form of dissolved carbonates in seawater at usual pH) into calcium carbonate is a dismutation reaction that liberates a stoichiometric amount of carbonic acid in the water column. This carbonic acid, together with the CO_2 produced by the respiration of sea urchins, algae, and bacteria in the rearing structures, tends to reach rapidly undesired levels in a large-scale intensive cultivation. We have observed sea urchins whose skeleton growth was totally inhibited in these conditions. CO_2 partial pressure was recorded to be 5 to 9 times higher than usual in seawater (despite strong aeration of the water) and was presumed to be the direct cause of the inhibition of the skeletogenesis.

These limitations force us to choose either a flow-through system or to provide a chemical filtration to level carbonates and carbonic acid concentrations. The present method could be considered as a semi-intensive, semiclosed system where both sea urchin densities and water renewals remain compatible with the equilibrium of the inorganic carbon in seawater without supplemental filtration. However, such a trade-off would not be compatible with a rearing strategy aiming to raise profit on a large scale.

For the moment, fresh algae used as food form part of the natural diet of *P. lividus*. The composition of this food is presumably correct, although it might not be necessarily optimal (Frantzis

and Grémare 1992, González et al. 1993, Fernandez and Boudouresque 1998). The major problem encountered with food is its stability once put in the rearing structures, because this echinoid, being a grazer, ingests it slowly. Uneaten food could easily give rise to undesired pollution. Hence, we recommend the use of a stable diet (*Enteromorpha linza*) in the present rearing method instead of higher quality algae (*Laminaria digitata*, *L. saccharina* Lamouroux or *Rhodomenia palmata* (L.) Greville, unpublished results) for juveniles. We also avoid using artificial diets at water temperature above 16°C without the presence of an efficient bio-filter in the rearing structures.

The use of fresh algae is not always possible or profitable on a large scale (Fernandez 1996). Hence, an artificial diet designed specifically for sea urchins seems necessary for intensified echiniculture and is presently under investigation by several authors (Fernandez and Caltagirone 1994, Klinger et al. 1994, Klinger et al. 1997, Klinger et al. 1998, de Jong-Westman et al. 1995a, de Jong-Westman et al. 1995b, Fernandez 1996). Results obtained so far are encouraging, especially in terms of GI, but the food we were able to test gave unsatisfactory results in terms of color and palatability of roe. Recent testing of semimoist diets on *Strongylocentrotus droebachiensis* Müller (Motnikar et al. 1997) seems to confirm the positive effect of the artificial diet on the gonadosomatic index and the failure to obtain high quality gonads in terms of color and taste. Trials with carotenoids-enriched artificial food to enhance the color do not yet produce high quality gonads (Goebel and Barker 1998). Thus, better formulation of the food is basic to achieve correct taste and color for exploitation.

Finally, we should mention that the rearing method described here is labor intensive. Hence, manpower cost could be too high when considering profit. This would require some adaptation or mechanization of the most time-consuming operations: feeding subadults and adults, cleaning the growth structures, grading the batches or extracting the gonads if sea urchins are not commercialized alive (exportation to Japan). However, these are technical problems that could be solved by the industry.

CONCLUSIONS

This rearing method constitutes a good working basis for design of a closed-cycle, land-based echiniculture. We suggest it

could be used as a standard method to evaluate improvement obtained by adaptations or modifications aimed at intensification or profitability of echiniculture. This method could possibly be adapted to other species, allowing better comparisons of the biology of respective species as well as their aquaculture potentials.

Latent remaining problems when scaling up and intensifying cultivation, aiming at raising profit, should not be regarded as unavoidable limitations, but should be considered as challenges to address in further studies. Being a "new" cultivated species, it is not surprising that these obstacles mostly concern less known life stages or "biological features or characteristics" of sea urchins: the transition between the endotrophic postlarva and the exotrophic juvenile, the mechanism of the intraspecific competition, the carbonate budget needed for skeletogenesis, and the biochemical pathways in gametogenesis and in stocking reserve material in the gonads. Thus, it is probable that advances in fundamental biology of echinoderms, and more particularly of echinoids, will suggest solutions to these problems in the future.

It would seem that further development of closed-cycle, land-based sea urchin cultivation is worthwhile and will undoubtedly promote diversification of aquaculture and production of high quality seafood. This will, secondarily, lead to conservation of the natural environment by limiting the fisheries impact on natural populations of sea urchins.

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EFFECT OF FOOD AVAILABILITY AND BODY SIZE ON OUT-OF-SEASON GONAD YIELD IN THE GREEN SEA URCHIN, *STRONGYLOCENTROTUS DROEBACHIENSIS*

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ABSTRACT The out-of-season gonad yield of the green sea urchin, *Strongylocentrotus droebachiensis*, was low in barren ground habitats, intermediate in kelp forest habitats, and high in laboratory tanks supplied with fresh kelp, *Laminaria hyperborea* and *L. digitata*. In August, the laboratory population had already attained gonad sizes comparable with the prespawning maximum of sea urchins from kelp habitats. Analysis of covariance (ANCOVA) suggested that both absolute gonad mass and relative gonad mass (gonad index) were increasing functions of urchin size. However, both cubic polynomial regression and graphical analyses detected a curvilinear relationship with a tendency toward decreasing gonad mass in the largest animals in the laboratory population and in the barren ground population. The tendency toward decreased gonad yield in the largest animals is consistent with a hypothesis of size-dependent reproductive senility. The practical implication for future echiniculture operations is that there seems to be a physiologically determined optimum size where gonad yield is maximized. The present data suggest that this optimum size is located in the 55 to 60 mm size interval. In this size interval, the laboratory population had approximately twofold larger gonad mass than the kelp population and approximately threefold larger gonad mass than the barren ground population.

KEY WORDS: *Strongylocentrotus droebachiensis*, gonad yield, resource allocation, sea urchin, echinoid, echiniculture, echinoculture

INTRODUCTION

Gonad yield is an important factor in the commercial exploitation of sea urchins. Previous studies have shown that the main determinants of gonad yield are body size (Gonor 1972), food availability (Hooper and Cuthbert 1994, Keats et al. 1983), and seasonal variation in reproductive condition (Byrne 1990, Himmelman 1978). Body size determines the maximum possible gonad size; whereas, food availability and reproductive condition determine the actual proportion of gonad biomass.

Kelp is a preferred natural food of exploited sea urchins (Larson et al. 1980), and habitats with an abundant supply of kelp provide the best gonad yield (Andrew 1986, Moore 1937). Such habitats are a limited resource, however (Mann 1973), and their distribution is further limited by dense sea urchin populations that can destroy large kelp forests and perpetuate a denuded habitat known as *barren grounds* in North America and *Isoyake* in Japan (Hagen 1983, Harrold and Pearse 1987).

Three strategies have been employed to improve the gonad yield of food-limited sea urchins from barren grounds: translocation, habitat improvement, and feeding (Hagen 1996a). In translocation, the sea urchins are captured 2 to 4 months before the harvesting season and released in a kelp habitat where the natural sea urchin density is low. Translocation is only feasible when the recapture rate is high and the cost of transportation and recapture is low.

Habitat improvement either consists of kelp reforestation in barren grounds (Kito et al. 1980) or the addition of artificial reefs in areas with soft or unstable bottoms (Saito 1992). Artificial reefs provide a stable substrate for kelp growth and are usually seeded with translocated urchins or hatchery-reared juvenile urchins that require 2 or 3 years before they reach harvestable size.

Feeding trials have been conducted on barren grounds, in bottom enclosures, in suspended cages, in laboratory tanks, and in land-based rearing facilities. Feeding with either fresh macroalgae or formulated feeds improves gonad yield (de Jong-Westman et al. 1995, Fernandez et al. 1995, Keats et al. 1983, Walker et al. 1998), however, the quality of the gonads may be adversely affected by

formulated diets deficient in carotenoids (Goebel and Barker 1998) or containing too much fish meal or too little kelp (Hooper et al. 1996, Hoshikawa 1993, Klinger et al. 1998).

Gonad yield is strongly influenced by the seasonal reproductive cycle of the sea urchin. A high proportion of the gonad biomass is released as gametes during the spawning season (Thompson 1984), making the gonads too small to harvest for several months thereafter. The harvesting season commences when gonad yield exceeds 10% of the total wet mass and lasts until quality starts to deteriorate as the gonads ripen prior to spawning.

The green sea urchin, *Strongylocentrotus droebachiensis*, produces high quality gonads when the availability of fresh kelp is adequate (Kramer and Nordin 1979). This species is currently being exploited in the Northwest Atlantic and, to a lesser extent, in the Northeast Pacific and the Northeast Atlantic. It is also a target species for the development of commercial echiniculture (Hagen 1990, Hagen 1996a). The spawning season of *S. droebachiensis* is in the spring (Cocanour and Allen 1967, Falk-Petersen and Lønning 1983, Himmelman 1978, Meidel and Scheibling 1998, Oganessian 1998), and the best harvesting period is in late autumn and early winter (Campbell and Harbo 1991).

The objectives of this study are: (1) to quantify the out-of-season gonad yield of *S. droebachiensis* in natural habitats with high and low food availability, and in laboratory tanks with a high availability of kelp, the urchin's preferred natural food; and (2) to quantify the effect of body size on the gonad yield.

MATERIALS AND METHODS

A total of 605 sea urchins were sampled from three different habitats: 85 urchins from a barren ground habitat at Værøy, an exposed island at the tip of the Lofoten Archipelago (Hagen 1995a); 383 urchins pooled from subsamples collected from four different *Laminaria hyperborea* kelp forests (three from Værøy; plus one other, see below); and 137 urchins pooled from eight different laboratory tanks. The datasets were pooled from subsamples to minimize spurious temporal or spatial variation.

The barren ground sample, and three of the kelp forest samples

TABLE 1.

ANCOVA analysis of the effect of habitat-related food availability (barren ground, kelp forest, laboratory tanks) on out-of-season gonad yield of *Strongylocentrotus droebachiensis*. The covariate is test diameter. The gonad yield (wet mass (g)) is cube root transformed.

Treatment	Df	SS	MS	F-Value	p-Value
Habitat	2	0.41	0.20	4.3	.01
Covariate	1	61.46	61.46	1305.4	.0001
Habitat · covariate	2	0.05	0.02	0.5	.6088
Residual	599	28.20	0.05		
Scheffe's post hoc Test		Difference	Critical Difference		p-Value
Barren ground vs. kelp forest		0.339	0.079		.001
Barren ground vs. lab tanks		0.672	0.091		.001
Kelp forest vs. lab. tanks		0.333	0.066		.001

from Væroy ($n = 111, 115, 134$ urchins), were collected in August 1992 as part of an ongoing ecological field study (Hagen 1983, Hagen 1995b). The kelp forest at Væroy had been overgrazed in the past, but there were no destructive grazing fronts at any of the field sites when the samples were made (Hagen 1995a). The exact location of the study sites is described by Hagen (1987). One kelp forest sample was collected outside The Marine Laboratory of Bodø College in a kelp forest habitat that provided the source population for the laboratory population ($n = 23$ urchins, $67^{\circ} 16'30''N$, $14^{\circ} 16'30'' E$). All field collections were made by SCUBA divers in shallow water (≤ 10 m deep).

Laboratory studies were initiated on March 3, 1993. The laboratory population was kept in running seawater tanks equipped with airstones. The water depth was 25 cm in four tanks and 50 cm in the other four, and the interior surface dimension was approximately 1×1 m² for all tanks. The experimental animals were fed *ad libitum* on kelp, mostly *L. hyperborea* and *L. digitata*, and occasionally *L. saccharina* and *Alaria esculenta*. The laboratory population was tagged with internal PIT (passive induced transponder) tags (Hagen 1996b). These tags did not seem to impede gonad growth, thus confirming their suitability for individual identification of echinoid broodstock. The density of the laboratory population was maintained at approximately 50 individuals/tank by regular removal and replacement of dead individuals. The mean residence time in the tanks was 234 days, and the minimum residence time was 41 days, thus ensuring that all experimental animals had ample time to build up large gonads (Russell 1998).

The laboratory population and its source population (i.e., the fourth kelp forest subsample, see above), were both sampled in August 1995. Field samples were collected, and all urchins were measured and dissected, as described in a previous paper (Hagen 1992). Test diameter was recorded with 0.1-mm precision calipers, and the total wet mass and the gonad wet mass, were recorded with 0.1 g precision electronic laboratory scales. Only urchins ≥ 30 mm in test diameter which were not infected with the nematode *Echinomermella matsi* (Jones and Hagen 1987), were included in the datasets. A gonad index was calculated as $100 \cdot \text{gonad wet mass} / \text{total wet mass}$.

Analysis of covariance (ANCOVA) was carried out using test diameter as the covariate and gonad wet mass as the dependent variable (Sokal and Rohlf 1995). The dependent variable was third root transformed prior to analysis. Residual plots did not reveal any gross violations of the assumptions of the analysis, indicating that the transformation had successfully linearized the relationship between covariate and dependent variable. Backtransformed esti-

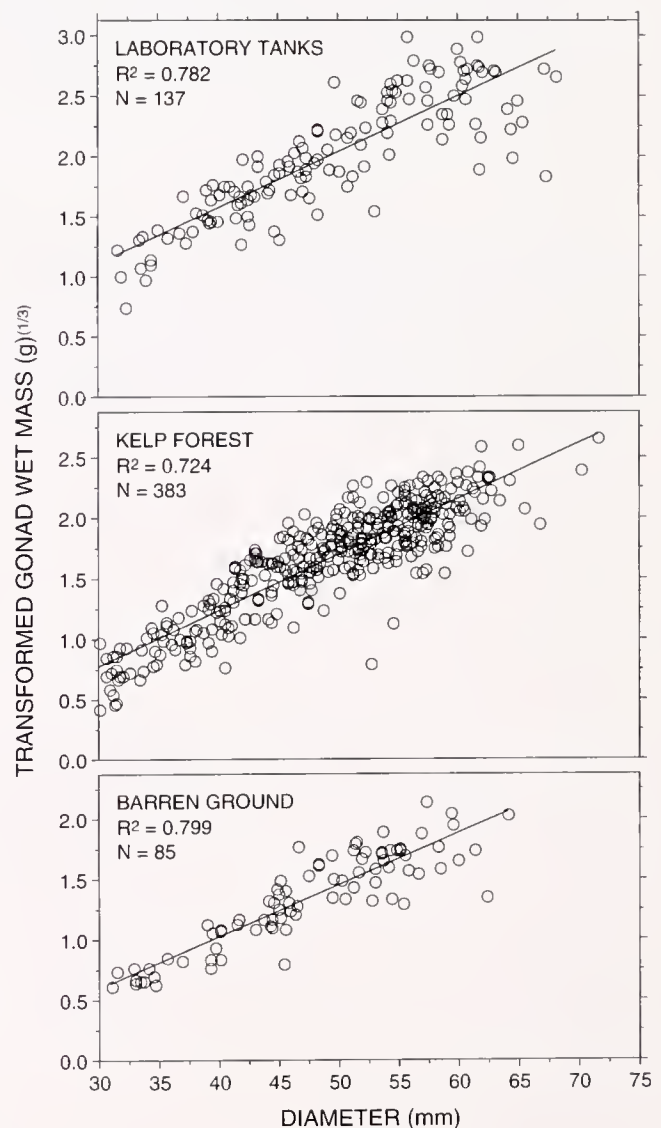


Figure 1. Regression lines describing the relationship between the test diameter and transformed gonad wet mass of *Strongylocentrotus droebachiensis* from laboratory tanks and two natural habitats with different food availability. The regression equations are: $Y_{\text{Tanks}} = -0.279 + 0.046 X$; $Y_{\text{Kelp}} = -0.597 + 0.046 X$; $Y_{\text{Barren}} = -0.697 + 0.043 X$.

mates of gonad mass were obtained for each urchin by cubing the results from the regression equation. The corresponding gonad index was estimated as $100 \cdot$ backtransformed gonad mass estimate/total wet mass.

RESULTS

Habitat-Related Food Availability

The interaction effect between habitat (barren ground, kelp forest, laboratory tanks) and covariate (test diameter) was not significant (Table 1), indicating that the regression lines in Figure 1 are parallel. The significant main factor effect, and *post hoc* tests, indicate that the lines have different intercepts; that is, the gonad size of urchins with similar size is low in the barren ground habitat, intermediate in the kelp habitat, and high in the laboratory tanks. The effect of the covariate is also significant, indicating that gonad size increases with increasing test diameter in all three habitats (Fig. 1).

The backtransformed regression curves confirm that gonad size increases with increasing test diameter (Fig. 2a), and the corresponding gonad index estimates suggest that the gonad index also increases with increasing urchin size (Fig. 2b). This result implies that gonad mass continues to increase at a faster rate than the over-all mass of the urchin throughout the entire observed size range.

Re-examination of the Data

The residual plot of the ANCOVA does not indicate any gross violations of the assumptions of the analysis (Fig. 3a). There is,

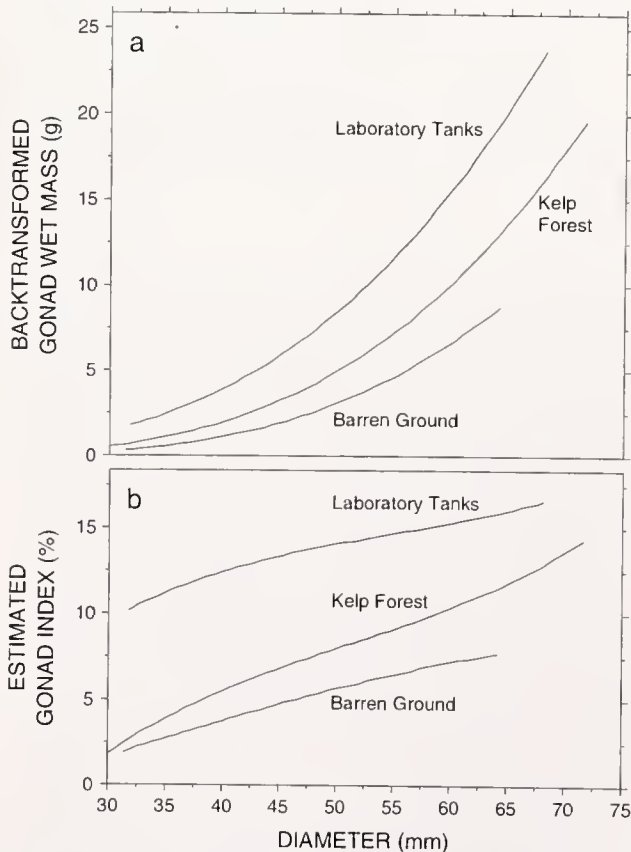


Figure 2. *Strongylocentrotus droebachiensis*. Backtransformed regression estimates. (a) Gonad wet mass; (b) The corresponding estimates of gonad index.

however, a slight dominance of positive residuals in the intermediate size range and a slight dominance of negative residuals at either extreme. This pattern is an indication of a curvilinear trend, which becomes more obvious when the residuals are plotted as size interval means (Fig. 3b). The plots suggest that the ANCOVA analysis has underestimated gonad yield in the intermediate size group and overestimated the yield for small and large urchins. This pattern is particularly prominent for urchins from the laboratory tanks.

The curvilinear pattern is also evident in the cubic regression curves of the untransformed gonad wet mass and gonad index data from the barren ground and laboratory tanks (Figs. 4, 5). Nevertheless, the gonad wet mass curve for the kelp forest habitat continues to increase, although the rate of increase is sufficiently low to stabilize the gonad index curve after an initial increase (Fig. 5). This pattern is confirmed by the size interval plot (Fig. 6a, b), which suggests that the highest gonad yield is attained by 55 to 60 mm urchins from laboratory tanks.

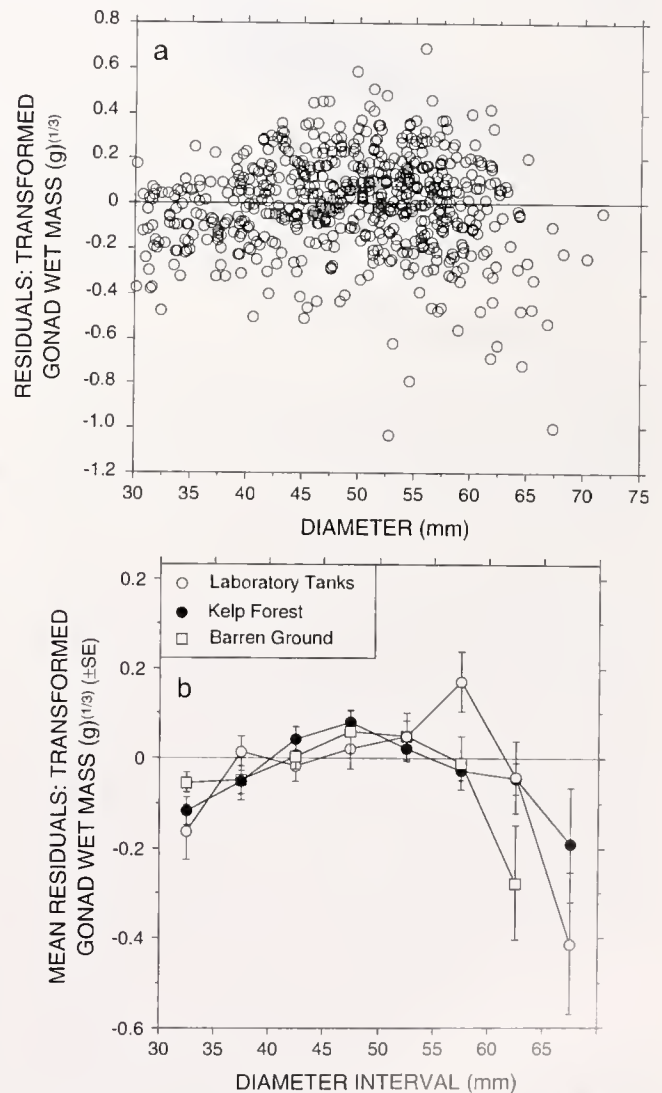


Figure 3. *Strongylocentrotus droebachiensis*. Residual plots for the ANCOVA. (a) Regression line describing the relationship between residuals and covariate; the regression equation is: $Y = 0.0001 - 0.000002 X$, $R^2 = 0$; (b) residual means for each 5-mm size interval of the observed size range.

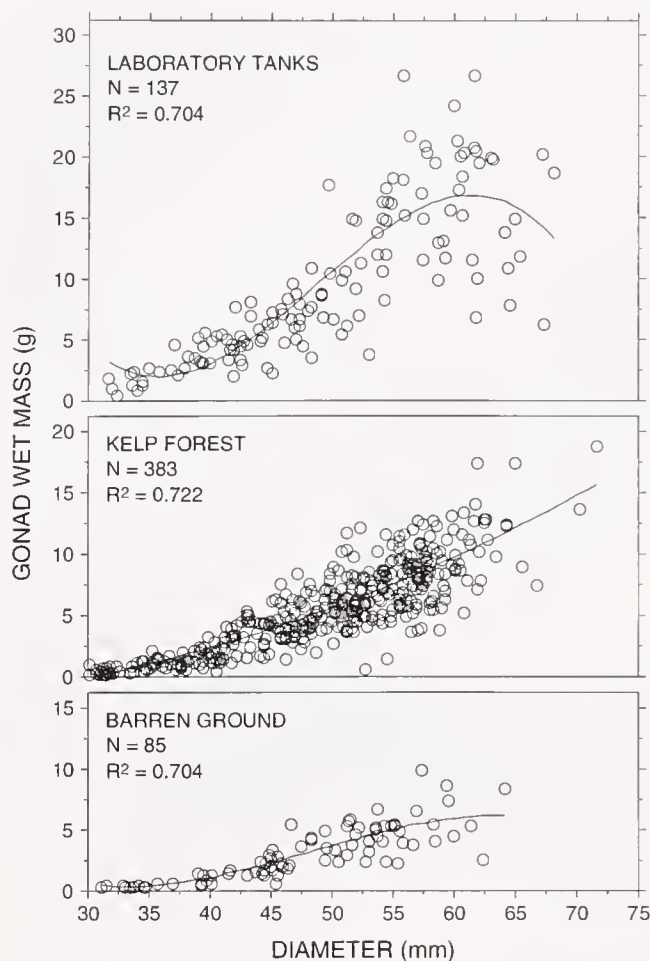


Figure 4. *Strongylocentrotus droebachiensis*. Cubic regression curves for the relationship between test diameter and untransformed gonad wet mass.

DISCUSSION

In August, the laboratory population had already attained gonad sizes comparable with the prespawning maximum of sea urchins from natural habitats (Himmelman 1978). Analysis of covariance indicated that gonad size increased with urchin size and that the gonad size of urchins of a similar size was high in the laboratory tanks, intermediate in the kelp habitat, and low in the barren ground habitat (Fig. 1). This simple and straightforward result is based on an assumption of random residual variation (Sokal and Rohlf 1995). Whether the assumption is accepted or rejected is largely a subjective decision, based on an apparent lack of obvious departures from randomness in the residual plot (Fig. 3a). In general, acceptance is at the risk of neglecting potentially useful information obtainable from the observed extremes of the data, where there are commonly a limited number of observations (Scharf et al. 1998). However, in this particular case, close scrutiny of the residuals suggests that the ANCOVA has underestimated gonad yield in the intermediate size range and overestimated the yield for small and large urchins. This is an indication that there may be an underlying nonlinearity in the data that is not accounted for in the covariance model, and a re-examination, using graphic analysis and cubic regression of the untransformed data does, indeed, reveal a more complex pattern.

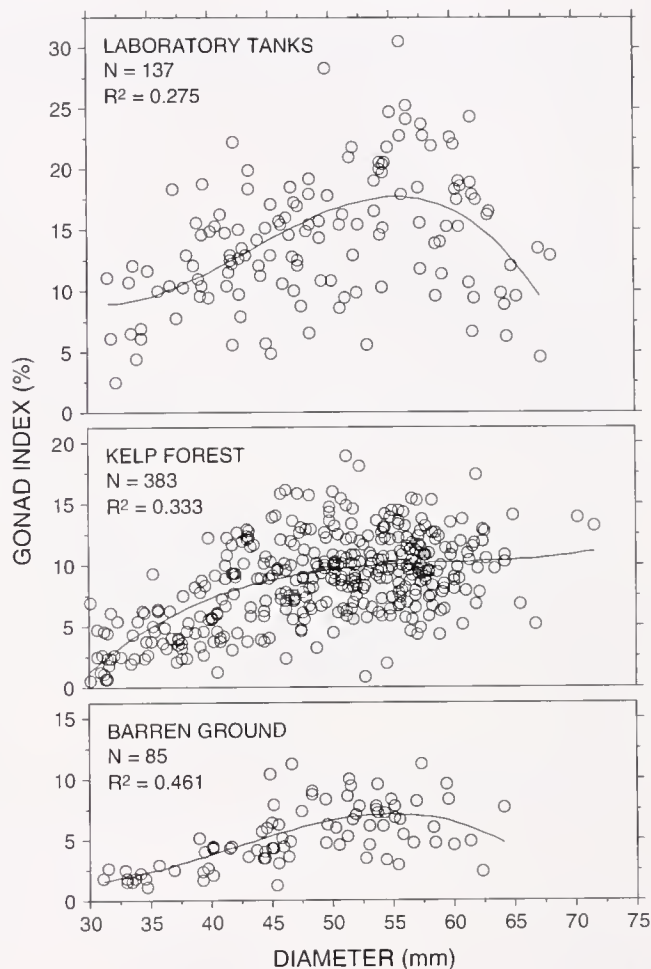


Figure 5. *Strongylocentrotus droebachiensis*. Cubic regression curves for the relationship between test diameter and untransformed gonad index data.

The revised analysis suggests that the mean gonad mass of urchins in the laboratory population increased to a maximum level of 17 g wet mass at a test diameter of approximately 60 mm and then stagnated and decreased for the largest urchins. The practical implication of this result for future echiniculture operations is that there seems to be a physiologically determined optimum size where gonad yield is maximized. The present data suggest that the optimum size is located in the 55 to 60 mm size interval. In this size interval, the gonad yield of the laboratory population was approximately twice that of the kelp forest population and approximately thrice that of the barren ground population.

The observed tendency toward decreased gonad yield in the largest animals may be an indication of size-dependent reproductive senility and is consistent with findings for three other echinoids (Gonor 1972, McShane and Anderson 1997, Moore et al. 1963). It has apparently not been detected previously for *S. droebachiensis*, although some data from the literature do exhibit a similar trend when re-analyzed (Fig. 7). The shape of the curves based on Kramer and Nordin's (1978) data for November match the shape, but not the peak, of the corresponding curves for the laboratory population (Figs. 6, 7). Whether this discrepancy is caused by environmental differences or by genetic differences between populations from the North Atlantic and the North Pacific is less important than the implication that the largest animals that any

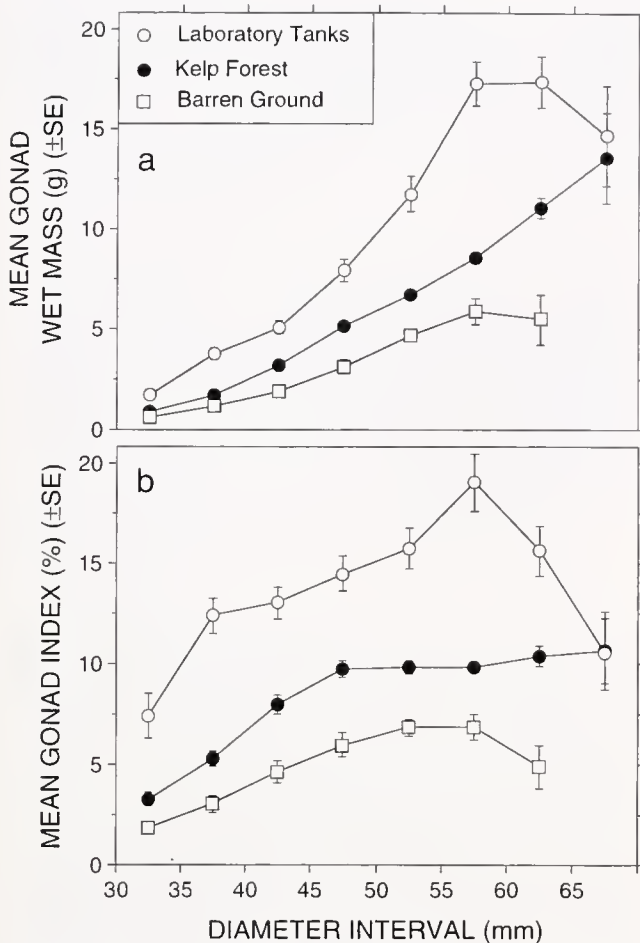


Figure 6. *Strongylocentrotus droebachiensis*. Size interval plots: (a) untransformed gonad wet mass; (b) gonad index.

given habitat will support must be sampled to detect a curvilinear pattern.

There was no tendency toward decreased gonad mass in large urchins in the kelp forest population, in Kramer and Nordin's (1978) data from August or in Miller and Mann's (1973) data, also from August (Figs. 6a, 7a). The corresponding gonad index curves did, however, reveal a tendency toward stagnation or a slight decrease, and it is possible that samples including larger animals would have detected a curvilinear pattern. An alternative explanation is that large urchins, which grow exceedingly slowly, may be able to allocate more resources to reproduction during the summer season when small and medium-sized urchins allocate resources to somatic growth. As resource allocation shifts from somatic to reproductive growth during the fall, the largest urchins may be less effective than smaller urchins because of the higher maintenance requirements of a larger biomass. The same line of reasoning would suggest that the curvilinear pattern of the barren ground population in August may be caused by resource limitations that would severely inhibit both somatic and reproductive growth. This scenario implies that to detect a curvilinear gonad yield pattern, a habitat with ample food supply should be sampled at the peak of the annual gonad cycle, before the female urchins reach full stage IV maturity (Byrne 1990), because later sampling is subject to sampling error caused by oozing of mature gametes from the go-

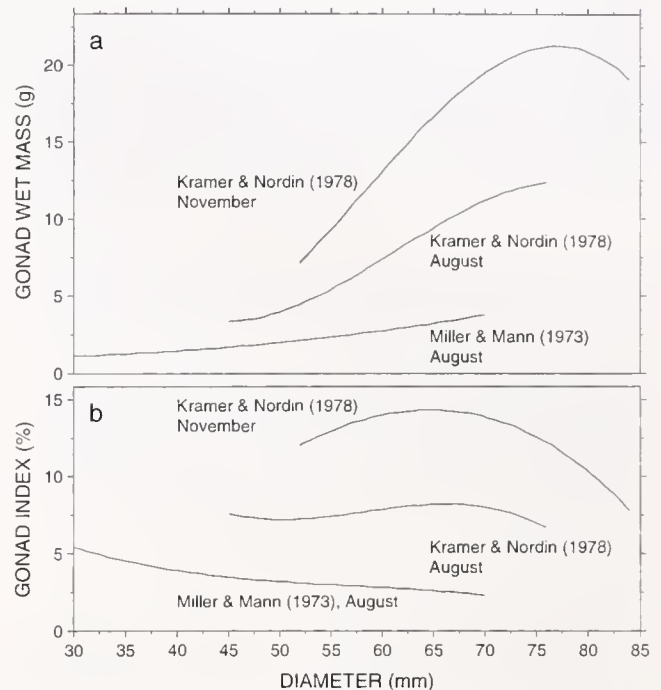


Figure 7. *Strongylocentrotus droebachiensis*. Cubic regression curves for data from the literature: (a) untransformed gonad wet mass; (b) gonad index; Kramer and Nordin (1978), based on raw data; Miller and Mann (1973), based on regression coefficients.

nads during sampling and increased variation caused by early spawning (Munk 1992).

Asynchronous or prolonged spawning, where low numbers of urchins may spawn in the summer or fall (Keats et al. 1987, Meidel and Scheibling 1998, Miller and Mann 1973), is a potential cause of variation in out-of-season samples from both field and laboratory (Fig. 4). Preliminary results suggest that this source of variation may be reduced or eliminated in future studies by providing a controlled laboratory or aquaculture environment that inhibits the formation of mature gametes (Hagen 1997, Walker et al. 1998).

The gonad index curve, based on Miller and Mann's (1973) regression coefficients for urchins collected in barren patches adjacent to a kelp forest, is atypical in that it exhibits a steady decline (Fig. 7b). This may be an artifact caused by approximations in the published regression coefficients. Reports that the gonad size of *S. droebachiensis* increases steadily with age (Lang and Mann 1976, Thompson 1984) should also be interpreted with caution, because, as this paper shows, a curvilinear trend may be easily overlooked in out-of-season samples with few large urchins (Lang and Mann 1976) or when gonad mass data are transformed to fit a linear model (Thompson 1984). The last argument also applies to Fuji's (1967) regression lines for *S. intermedius*.

In conclusion, the present study suggests that the effect of gonad enhancement efforts is maximized for green sea urchins of intermediate size because of an easily overlooked tendency toward decreasing gonad size in large urchins. In addition, it shows that gonad yield can be enhanced by maintaining green sea urchins in a protected environment with an abundant supply of kelp, their preferred food, so that gonads with acceptable market size can be produced several months before the harvesting season of natural populations.

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AQUACULTURE OF THE SEA URCHIN (*STRONGYLOCENTROTUS NUDUS*) TRANSPLANTED FROM CORALLINE FLATS IN HOKKAIDO, JAPAN

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ABSTRACT To establish aquaculture of the sea urchin *Strongylocentrotus nudus* on coralline flats where no commercial harvest had been previously obtained, a field experiment on gonadal growth was carried out in a dredged area at Matsumae in southern Hokkaido from October 1988 to March 1989. *S. nudus* taken from coralline flats and fed on the flesh of the fish Pacific saury *Cololabis saira* and arabesque greenling *Pleurogrammus azonus* from October to January showed markedly enhanced gonadal growth. The gonadal moisture and glycogen contents increased and decreased, respectively, and the gonadal whiteness and chroma increased and decreased, respectively. However, all these parameters, excluding the gonadal whiteness, improved after changing the diet to the brown algae *Laminaria japonica* and *Undaria pinnatifida* from February to March. I concluded that, after feeding sea urchins on fish, active algal grazing must be maintained at the culture ground to improve the gonadal quality.

KEY WORDS: Sea urchin, *Strongylocentrotus nudus*, aquaculture, gonadal growth, feeding

INTRODUCTION

The sea urchin *Strongylocentrotus nudus* is found on intertidal and subtidal rocky sea bottoms. It is distributed from Dalian in China to Primorskyi Krai in Russia and in Japan. It is found in the Pacific Ocean from Sagami Bay to Cape Erimo, Hokkaido, and in the Sea of Japan from Oumi Island to Soya Cape (Kawamura 1993). This sea urchin is harvested commercially in regions of Tohoku and Hokkaido (Imai 1995, Agatsuma 1991). Landing of *S. nudus* accounts for about 44% of the six species harvested commercially in Japan (the other five are *S. intermedius*, *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus*, *Anthocidaris crassispina*, and *Tripneustes gratilla*).

Various fishery management policies, such as the establishment of a minimum catch size and a closed season for fishing to allow natural reproduction and stock enhancement by such means as sea urchin transplantation and the introduction of artificial stone beds to enhance algal growth have been implemented (Agatsuma 1991). However, commercial harvests of sea urchins fluctuate annually, and spatial variations are marked (Agatsuma 1997). Therefore, a stable fishery has not yet been established.

In the subtidal zone of the Sea of Japan off the coast of southwestern Hokkaido, the population of *S. nudus* is dense, >7 individuals/m², and it is the dominant species among the benthic animals distributed on coralline flats where only crustose coralline red algae grow (Fujita 1989, Nabata et al. 1992, Agatsuma et al. 1997). Coralline flats in various parts of the world have been reported to be maintained by intensive grazing of sea urchins (North 1971, Lawrence and Sammarco 1982, Harrold and Pearse 1987). However, the maintenance of coralline flats leads to reduced production by fisheries; this reduction is called *Isoyake* in Japanese (Taniguchi 1996). In various parts of the world, removal of sea urchins from coralline flats has resulted in colonization of the flats by large algal populations (Kikuchi and Uki 1981, Sawada et al. 1981, Harrold and Pearse 1987, North 1971, Yotsui and Maesako 1993). In recent years, the experimental removal of densely distributed *S. nudus* from coralline flats in the Sea of Japan off the coast of southwestern Hokkaido has resulted in colonization by the large perennial brown alga *Sargassum confusum* (Agatsuma et al. 1997). This finding led to the conclusion that the temporal and spatial

distributions of coralline flats in this area were also maintained by intensive grazing by these sea urchins. The growth rate of these sea urchins was extremely low, and their gonads were undeveloped (Agatsuma et al. 1997), so they were not harvested. However, because these sea urchins are an important fishery resource, the establishment of an aquaculture system to enhance their gonadal production would be of commercial value.

The cultured kelp *Laminaria japonica* is a valuable diet for sea urchins (Sato and Notoya 1988), and yields of the kelps are high during spring and summer, when the gonadal indices of sea urchins fed this kelp in excess were found to reach 18% (the minimum level for commercial harvesting) for about 2 months (Agatsuma 1997). The gonadal indices of sea urchins at traditional fishing grounds during winter and spring are only about half the maximum level in summer (Agatsuma et al. 1988, Agatsuma et al. 1989). Therefore, it is considered that promotion of the gonadal growth of *S. nudus* by transplanting a dense population of these sea urchins to an area in a maintained fishing ground will contribute to improving the commercial harvest.

The diet for *S. nudus* comprises algae (Kawamura 1966) and the animals, *Mytilus edulis*, *Balanus* spp., and *Celleporina* spp. (Kittaka 1977, Kittaka and Imamura 1981). Artificial diets containing protein are effective for increasing the gonadal size of sea urchins (Levin and Naidenko 1987, Lawrence et al. 1992, de Jong-Westman et al. 1995). At Aomori Prefecture in the northernmost part of the Tohoku region of Japan, experiments on hanging aquaculture of sea urchins fed fish were conducted. The results showed that the gonads of sea urchins that fed on fish grew rapidly, but their color and taste became poor. Subsequently, algal food was supplied after feeding the urchins fish in an attempt to improve the gonadal quality (Uemura et al. 1986). However, changes in the chemical composition of the gonads of sea urchins fed fish and then algae have not been elucidated. Furthermore, the number of sea urchins that can be cultured by the hanging method is too low to be commercially viable.

In this study, I transplanted *S. nudus* from a dense population living on coralline flats to a dredged area, fed them on fish and then algae from winter to spring and examined the sizes and changes in the chemical composition of the gonads.

MATERIALS AND METHODS

One thousand individuals of *S. nudus* (test diameters >40 mm) that inhabited coralline flats at depths of 8 to 15 m were collected by scuba diving in the Sea of Japan off the coast of Satsumae in Matsumae, southern Hokkaido on October 5, 1988. Five hundred were immediately transplanted to two experimental turtle shell-shaped blocks, which were covered with fishing nets, at a depth of 2 m in a dredged area (Fig. 1A, B). Before transplantation, the test diameters (mm) of 300 individuals sampled at random were measured using slide calipers. After transplantation, the gonads of 10 individuals were examined with the naked eye every 7 days, and 1 confirmed that spawning had finished by October 15.

To examine the effect on the gonadal size of feeding the sea urchins two fish species, groups A and B were fed with 5 to 7 kg Pacific saury (*Cololabis saira*) or arabesque greening (*Pleurogrammus azonus*), respectively, every 7 to 12 days from October 19, 1988 to January 19, 1989. Then, the sea urchins at each experimental site were fed 3 to 30 kg kelp (*Laminaria japonica* and *Undaria pinnatifida*) every 3 to 13 days until March 25, 1989 (Table 1). A traditional fishing ground at the depths of 1 to 2 m, where the biomass of *Laminaria religiosa* is seasonally abundant during spring/summer, and high wave action is observed during December to February outside the dredged area was used as the control site (C). Every month, 20 sea urchins were collected from the experimental and control sites, and their test diameters, live wet weights (g) and gonadal wet weight (g) were measured, and the gonadal index (gonadal wet weight \times 100/body wet weight) was calculated. The ages of these sea urchins were determined by the method of Jensen (1969) and counted in full as the spawning in September (Agatsuma et al. 1988, Agatsuma et al. 1989). The surface water temperatures were measured with a mercury thermometer every 10 days at the dredged area and monthly, excluding October and December, at the control site.

TABLE 1.

Amount (kg) of foods fed on two experimental groups (A, B) of *Strongylocentrotus nudus*.

Date	A			B		
	C.s*1	L.j*2	U.p*3	P.a*4	L.j	U.p
October 19, 1988	5			6		
October 31	7			7		
November 8	6					
November 10				7		
November 16	5			5		
November 28	7			7		
December 19	7					
December 25				7		
December 31	7			7		
January 11, 1989	7			7		
January 19	7			7		
January 26		5			5	
February 10		3			3	
February 19			4			
February 27		10			10	
March 2		10			10	
March 8			30			30
March 21		5			10	
March 25		5			10	
Total	58	38	34	60	48	30

* *Cololabis saira*.

* *Laminaria japonica*.

* *Undaria pinnatifida*.

* *Pleurogrammus azonus*.

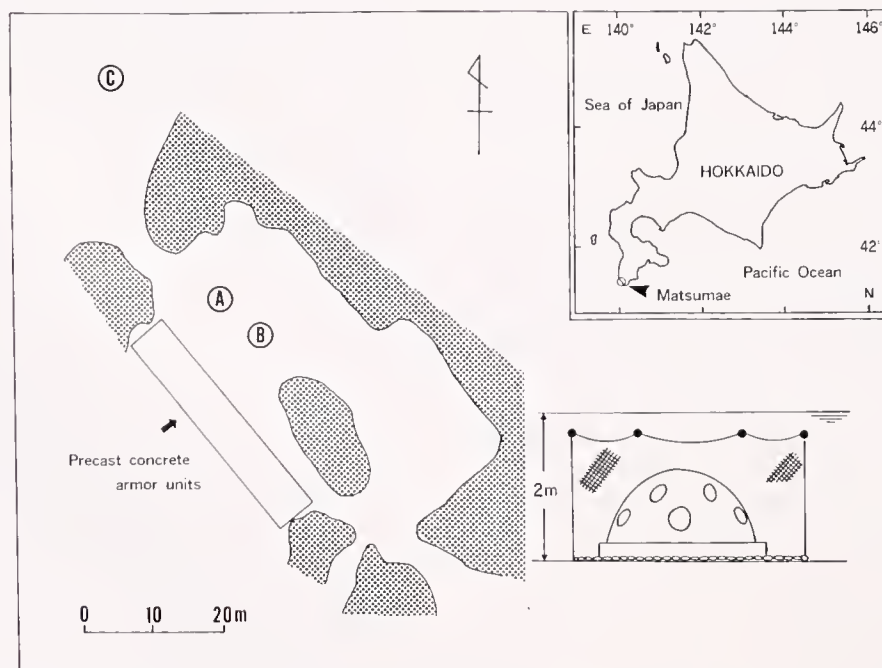


Figure 1. Map showing the area studied. Two experimental compartments (A, B) of turtle shell-shaped blocks were placed in a dredged area and covered by a fishing net, and the control site was in a traditional fishing ground (C).

To determine the serial changes in the proximate composition of the gonads of groups A, B, and C, the moisture, glycogen, crude protein, and crude fat contents were measured. The moisture content was determined by measuring the weight loss after drying in an air oven at 105°C for 16 h, and the glycogen content was determined by the anthron-sulfuric acid method of Roe (1955). The nitrogen content was determined by the micro Kjeldahl method, and the percentage of nitrogen was multiplied by 6.25 to estimate the crude protein content. Crude lipids were extracted with diethyl ether using a Soxhlet apparatus. The glycogen, crude protein, and crude lipid contents are expressed as mean percentages (dry weight for dry weight) of the gonadal matter. The gonadal chroma and whiteness were also measured using a colorimeter (Nippon Denshoku Co. Ltd., Tokyo). The chroma and whiteness of the gonads were determined using the CIE standard colorimeter system as follows

$$\text{Chroma} = \sqrt{a^2 + b^2}$$

$$\text{Degree of white color} = \sqrt{100 - (100 - L)^2 + a^2 + b^2}$$

where a, b, and L represent the hue, chroma, and lightness, respectively.

RESULTS

Water Temperature

The surface water temperature at the dredged area where groups A and B were cultured fell from 15.3°C on October 19, 1988 to 6.0 to 6.5°C during January and February 1989, reached its minimum level of 5.5°C on March 8, and rose to 7.6°C on March 27. The water temperature changes at the control site were similar to those at the experimental site (Fig. 2).

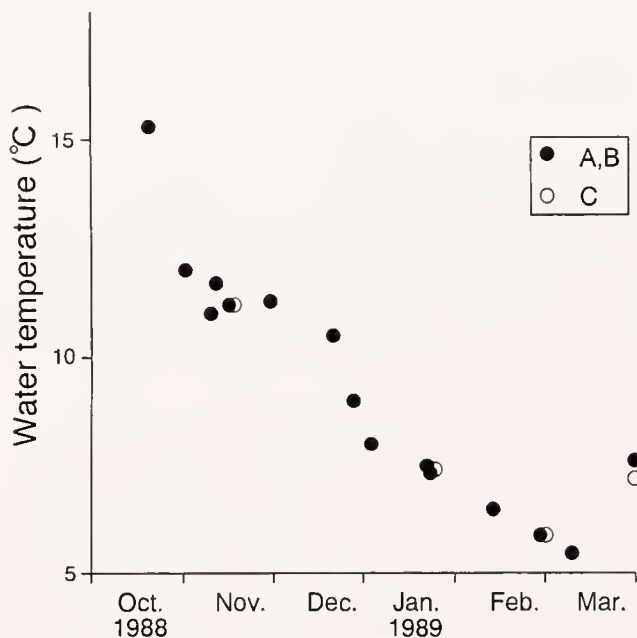


Figure 2. Variations in the surface seawater temperatures at the experimental and control sites.

Gonadal Growth

The initial test diameters of *S. nudus* transplanted to the experimental sites ranged from 42 to 74 mm. In particular, large numbers of sea urchins with test diameters of 50 to 60 mm were found (Fig. 3). Most of these were 5-year-old and secondarily 6-year-old sea urchins (Table 2).

The initial average gonadal indices (Fig. 4) of the sea urchins on October 19, 1988 were 5.8 for group A and 5.7 for groups B and C. The gonadal index of group A increased rapidly after feeding on *C. saira* and continued to increase after late January, when the diet was changed to *L. japonica* and *U. pinnatifida*, reaching 23.1 on February 27, after which, it declined slightly to 21.8 on March 27. The gonadal index of group B, which was fed on *P. azonus*, increased until December and then remained constant until late January, after which, it increased again when they were fed algae and it reached 21.8, identical to the group A value, on March 27. The gonadal index of group C increased until late December, remained unchanged until late February, when the water temperature fell to 6.0 to 6.5°C, and then increased again to 17.8 on March 27. The difference between the gonadal indices of the experimental groups and a control group during January and March was found to be statistically significant (Mann-Whitney U-test, <0.01).

Changes in the Chemical Composition of the Gonads

The initial moisture contents of all the groups (Fig. 5A) were 66%, although those of groups A and B increased to 77% and 74%, respectively, when they were fed on fish and remained high during this feeding period. After late January, when the diet was changed to algae, it declined gradually. The moisture content range of group C was 65 to 69 % throughout the experiment.

The initial gonadal glycogen contents (Fig. 5B) of all three groups were 35.7 to 39.9 %. After feeding on fish, a marked decline to 19.9 % and 24.8 % was observed in groups A and B, respectively. This decline continued until late February, by which

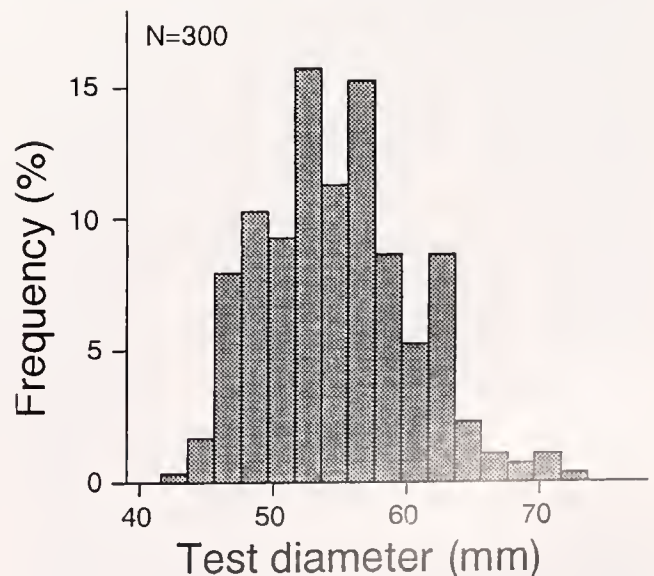


Figure 3. Size frequency distributions of *Strongylocentrotus nudus* transplanted to the two experimental blocks (A, B) on October 5, 1988.

TABLE 2.

Test diameter (\pm SE) and age composition of three experimental groups (A, B, C) of *Strongylocentrotus nudus*.

Groups	Date	No. Examined	Test Diameter	Age Composition					
				IV	V	VI	VII	VIII	IX
A	October 19, 1988	20	55.9 \pm 7.2	2	12	5	1		
	November 16	20	58.7 \pm 9.3		11	3	3	3	
	December 19	20	53.4 \pm 8.2		15	2	2		1
	January 20, 1989	20	57.5 \pm 5.4		7	8	1	2	2
	February 27	19	52.6 \pm 4.8		14	4	1		
	March 27	20	56.8 \pm 6.4	1	13	2	2	2	
B	October 19, 1988	20	53.7 \pm 4.6	1	10	8	1		
	November 16	20	57.4 \pm 7.1		10	5	3	2	
	December 19	20	57.9 \pm 5.7		9	4	3	3	1
	January 20, 1989	19	55.3 \pm 4.8		10	7		1	1
	February 27	20	58.2 \pm 6.5	1	6	6	5	2	
	March 27	20	55.4 \pm 4.4	1	9	7	2	1	
C	October 19, 1988	20	53.7 \pm 4.6	1	10	8	1		
	November 16	20	56.8 \pm 4.4	2	10	6	1	1	
	December 19	13	58.9 \pm 6.3		6		3	2	2
	January 20, 1989	20	54.5 \pm 4.2		15	4	1		
	February 27	20	57.0 \pm 4.5		13	6		1	
	March 27	20	54.8 \pm 5.4		16	4			

Group A, was supplied the meat of pacific saury and the kelps *Laminaria japonica* and *Undaria pinnatifida*; group B, was supplied the meat of arabesque greenling and kelps; group C, native control population

time they were feeding on algae. By late March, the gonadal glycogen content of group A did not decrease further; whereas, that of group B increased. The gonadal glycogen content of group C increased gradually during October and November, then rapidly to 55.7 % by late January, decreased in late February and then increased slightly in late March. The gonadal glycogen content of this group was >32 % throughout this experiment (Fig. 5 (B)).

The initial gonadal crude fat contents (Fig. 5C) of the three groups were 22.7 to 22.9 %. They decreased gradually until December or January, then those of group B and C gradually in-

creased after late January. Therefore, only very slight changes in the crude fat contents of the three groups were observed during this experiment.

The gonadal crude protein contents (Fig. 5D) of the three groups increased from the initial values of 24.5 to 33.6 % to 41.4 to 54.3 % until late February and then decreased slightly until late March. The gonadal crude protein contents of groups A and B during November and March were higher than those of group C.

The chroma of the gonads (Fig. 6A) of groups A and B decreased to minimum values on December or January, increased after late January, when the diet was changed from fish to algae, and reached a value similar to that of group C in late March. The chroma of group C decreased transiently to 21.6 by late December, but it ranged from 24 to 25 and was higher than the group A and B values during the other months.

The gonadal whiteness (Fig. 6B) increased after the sea urchins were fed on fish. In particular, the group A value increased rapidly from its initial value of 34 to 44 in late February. The group B value increased from its initial value of 36 to 41 by late November, then remained constant until late March. The whiteness range of group C was 32 to 36, and no marked changes were observed during the study.

DISCUSSION

During January and February, the gonads of *S. nudus* at the traditional fishing grounds in the Sea of Japan and Tsugaru Straits off the coast of southern Hokkaido are in the developmental stages of recovery and growth, and the gonadal index is low, about 10 % (Agatsuma et al. 1988, Agatsuma et al. 1989). The feeding rate on kelp of *S. nudus* decreases when the water temperature is low, and the daily feeding rate of the sea urchins with test diameters of 48.3 mm was 1.1 % when the temperature was 3.0 to 3.5°C (Machigu-

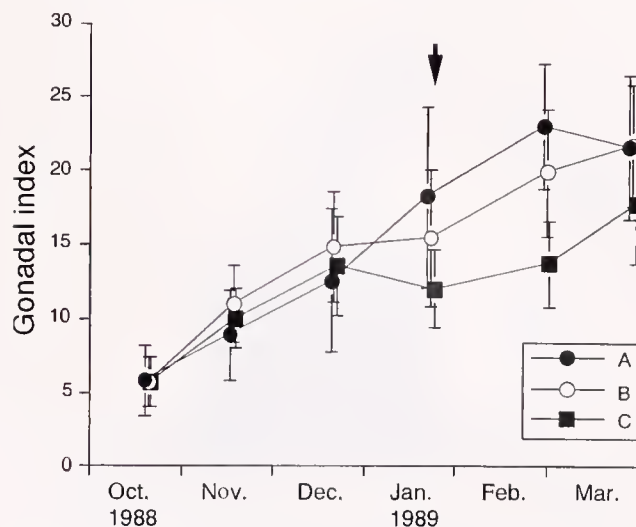


Figure 4. Monthly changes in the gonadal indices of the two experimental and one control group of *Strongylocentrotus nudus*. The arrow indicates the time when the food of groups A and B was changed from fish to algae. Each vertical bar represents standard deviation.

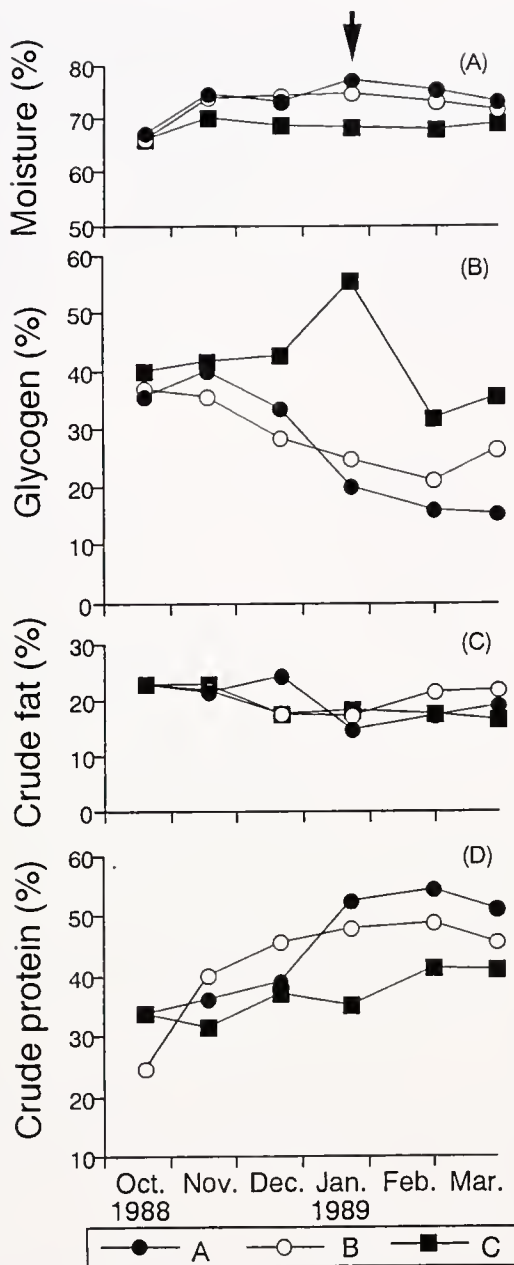


Figure 5. Monthly changes in the gonadal moisture, glycogen, crude fat, and crude protein contents of the three experimental groups of *Strongylocentrotus nudus*. The glycogen, crude fat, and crude protein contents are expressed as percentages (dry weight for dry weight) of the gonadal matter. The arrow indicates the time when the food of groups A and B was changed from fish to algae.

chi et al. 1994). The feeding rate, on a wet weight basis, when the sea urchins were fed on fish was 0.5 % when the water temperature was 5.0°C, lower than that when they fed on kelp, but their gonads grew well (Uemura et al. 1986). The feeding rate of the sea urchin *Loxechinus albus* fed on artificial feed containing menhaden meal was lower than that of those fed on algae, on a wet-weight, but not on a dry-weight, basis. However, the artificial feed supported gonadal growth better than the natural food (Lawrence et al. 1997). These findings indicate that the digestibility and rate of conversion to gonadal tissue of protein diets are high. Carbohydrates were

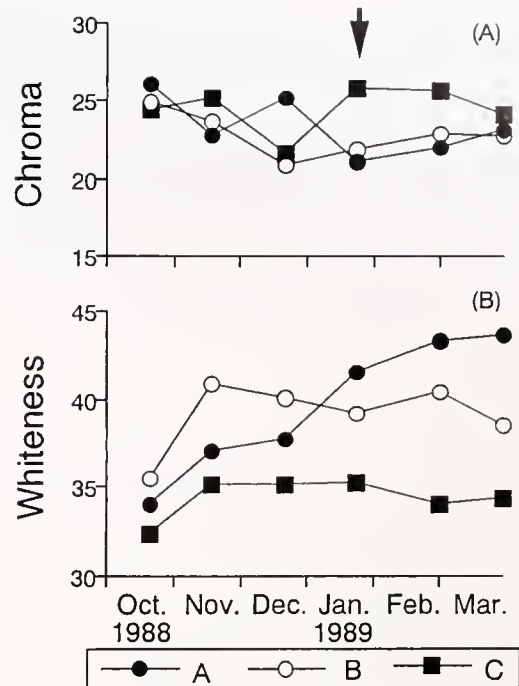


Figure 6. Monthly changes in the gonadal chroma and whiteness of the three experimental groups of *Strongylocentrotus nudus*. The arrow indicates the time when the food of groups A and B was changed from fish to algae.

found to be abundant in the gonads of *S. nudus* that graze only on kelp and glycogen was the major gonadal component (Hirano et al. 1978).

In this study, feeding on fish led to increases in the moisture content and whiteness and decreases in the glycogen content and chroma of the gonads of *S. nudus* (Figs. 5, 6). Nishikiori (1989) observed moisture content is less than 70 %, and glycogen content increases in the gonads of *S. nudus* fed *L. japonica* in excess from autumn to spring. Therefore, the chemical composition that accumulate in the gonads of *S. nudus* fed on protein and algal diets differ. β -carotene and β -echinenone derived from algae have been reported to be the major carotenoids in the gonads of sea urchins (Fox and Hopkins 1966), and it is well known that gonadal β -echinenone is derived from dietary β -carotene, via β -isocryptoxanthin (Griffiths and Perrott 1976). The principal carotenoid in marine red fish meat is astaxanthin (Tanaka et al. 1976), which is considered to affect the increase in the gonadal whiteness of *S. nudus* that fed on fish.

In this study, algal feeding after fish feeding led to decreases in the moisture content and increases in the glycogen content and chroma of the gonads (Figs. 5, 6). The particular flavor of sea urchin gonads is affected by the free amino acids glycine and alanine with sweet taste, which are abundant in the gonads of *S. nudus* that graze on kelp (Hirano et al. 1978). When the fish feeding period is long, the content of valine with a bitter taste increases, and glycine and alanine contents decrease. After changing the diet to kelp, glycine and alanine contents increase, when the fish feeding period is short (Hoshikawa et al. 1998). Therefore, to improve the gonadal quality, active grazing on algae of the sea urchins should be maintained after fish feeding. When the grazing activity of *S. nudus* is reduced by their gonadal development, low water temperature (Agatsuma et al. 1996), and high wave action

(Yano et al. 1994), gonadal development must not be promoted to excess by feeding algae during periods when the water temperature is low, <5°C. In the future, studies on the nutritional metabolism of sea urchins should be carried out to improve the gonadal quality after fish feeding, and to establish an aquaculture system involving fish feeding followed by algal feeding that promotes gonadal growth and improves gonadal quality. When sea urchins are cultured, grazing activity leads to gonadal growth and should be maintained. Therefore, they must be cultured in calm water, which is subject to moderate interchange with water from the open sea, to avoid the water temperature falling too much as a result of low air temperatures during winter.

The longevity of *S. nudus* is 14 or 15 years (Agatsuma 1991). Most of the sea urchins used in this experiment are 5 and 6 years old (Table 2), and their gonads evidently grew by fish and algal feeding. In the future, the range of ages of the sea urchins to be utilized in aquaculture also should be elucidated.

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SOMATIC AND GONADAL GROWTH OF THE SEA URCHIN *PSAMMECHINUS MILIARIS* (GMELIN) FED ARTIFICIAL SALMON FEED COMPARED WITH A MACROALGAL DIET

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ABSTRACT The effect on somatic growth of a commercially manufactured diet (salmon feed) compared to a macroalgal diet (*Ulva lactuca* or *Laminaria saccharina*) was studied for three stages in the life history of the green sea urchin, *Psammechinus miliaris* (Gmelin). Three size classes were studied; 0.89 to 1.1 mm, 15.3 to 15.8 mm and 21.3 to 21.7 mm. The effect on gonadal growth of the salmon feed compared to a macroalgae diet (*L. saccharina*) was also studied. A significant difference was observed in the somatic growth in the two largest size classes, with the sea urchins fed salmon feed exhibiting the highest growth rates. In the smallest size class however, there was no difference in the test diameter of the urchins fed the salmon feed and macroalgae at the end of the trial, although both had a significantly greater test diameter than the “unfed” control group. A significant increase was also seen in the gonadal index (GI) of the urchins fed salmon feed compared with the macroalgal diet. This growth was extremely rapid, with an increase in GI from 3.7% to 27.5% in 4 weeks, and there was no postspawning decrease in the GI observed. The experiments highlight the resource partitioning between somatic and gonadal production throughout the life history of *P. miliaris* in response to diet quality. The elevated production observed in *P. miliaris* fed the manufactured diet also suggests that this could be used as a basis for the development of a more refined diet for this potential aquaculture species.

KEY WORDS: Echinoidea, somatic growth, gonadal growth, artificial diet, *Psammechinus miliaris*

INTRODUCTION

Depletion of native, edible sea urchin populations caused by overfishing in many parts of the world has led to a growing interest in the cultivation of shallow water species and the identification of high quality diets. Somatic (test) and gonadal growth have been studied for a number of species of sea urchin fed a variety of algal species (Frantzis and Gremare 1992) and algal/vegetable-based artificial diets (Fernandez and Caltagirone 1994) in an attempt to assess the “quality” of these diets. The observation that echinoids in their natural habitat feed omnivorously on a variety of organisms (Lawrence 1975) has led to the suggestion that nonalgal foods can comprise a significant part of the diet of certain species (Nestler and Harris 1994). A number of studies have compared whether adult sea urchins exhibit a higher somatic growth rate on diets containing animal-derived proteins and lipids as compared to diets composed of either macroalgal or vegetable-derived materials, but no conclusive results have been obtained (Lawrence et al 1992, Fernandez and Boudouresque 1998).

In the case of gonadal growth, animal-based artificial diets have caused a significant increase in the gonad size in *Paracentrotus lividus* (Lawrence et al 1992) and *Strongylocentrotus intermedius* (Levin and Naidendo 1987) when compared to an vegetable-based artificial diet. Regardless of the dietary base, the majority of artificial diets have led to a significant increase in gonadal growth when compared to a purely macroalgal diet or an *in situ* diet (Fernandez et al. 1995), suggesting that the algal diet is lacking certain elements necessary for rapid gonadal growth.

Few studies, however, have been conducted on the nutritional requirements of sea urchins as they develop from postmetamorphosis to adulthood, with researchers generally concentrating on one developmental stage. In a study on “juvenile” sea urchins, with an initial test diameter of 0.3 to 0.5 mm, Cellario and Fenaux (1990) found that in *Paracentrotus lividus* the highest somatic growth occurred within the first 100 days after metamorphosis and

prior to gonadal production. In contrast, research on “adult” sea urchins has found that somatic growth rates tend to be reduced, and this has been attributed to the re-allocation of resources to gonadal growth (Lawrence et al. 1992, Klinger et al. 1994).

Psammechinus miliaris has been identified as a potential aquaculture species. This species has been found to reach sexually maturity at a test diameter of between 8 to 10 mm (Jensen 1969) and to exhibit significantly higher gonadal growth when maintained in cages stocked with the Atlantic salmon (*Salmo salar*) than when maintained in empty salmon cages or collected from the wild (Kelly et al. 1998). One possible reason for this enhanced gonadal growth may have been that the urchins were able to feed upon uneaten salmon pellets, which are extremely rich in animal-derived proteins and lipids. The objectives of this study were therefore, to determine whether somatic growth rates, throughout the life history of *P. miliaris*, from post-metamorphosis to adulthood, and gonadal growth rates were enhanced by salmon feed as compared to a “natural” macroalgal diet.

MATERIALS AND METHODS

The dietary experiments were undertaken at Dunstaffnage Marine Laboratory (DML) (56°28'30"N; 5°22'00"W). The animals used in the experiments were either reared in the hatchery at DML or collected using SCUBA from a sublittoral population (depth range 3 to 6 m) in Columbas Bay, Loch Creran (56°32'20"N; 5°17'00"W). All the sea urchins were starved for 2 weeks to ensure acclimatization and to achieve a similar nutritional status prior to the start of the experiment (Leighton 1966, Vadas 1977). There were three replicates of each treatment, and the urchins were fed *ad libitum*. The salmon pellets were replaced three times per week, and the macroalgae, without epiphytes, was replaced as required. The water temperature ranged from 4 to 16°C, and the mortality rate was recorded for all the experiments.

Somatic Growth

"Juvenile" (Test Diameter: 0.89 mm to 1.1 mm)

Twenty-five, hatchery reared *P. miliaris* (37 days old) were placed into each of nine cylindrical containers (0.1 × 0.1 m; with 40 µm mesh at each end). Each container was placed in a circular aquarium (10 L), which was supplied by sand-filtered seawater in a continuous flow through system with a flow rate of 300 ml min⁻¹. The urchins were fed either *Ulva lactuca*, a soft macroalgae on which this species is known to feed at this test diameter (Leighton 1995), or a commercially manufactured salmon feed (TROUW Crumbed Par Pellets). A control group had no additional feed. The horizontal test diameter for all the individuals was measured to the nearest 0.05 mm every 2 weeks using a dissection microscope or with the aid of dial callipers (Bedford and Moore 1985), depending on their size. The trial began in July 1997 and lasted for 6 months.

"Intermediate" (Test Diameter: 15.3 mm to 15.8 mm)

Twenty-five sea urchins were placed into nine compartments, within three rectangular tanks (0.7 × 0.6 × 0.3 m), which were supplied by a continuous flow through system of sand-filtered seawater with a flow rate of 1500 ml min⁻¹. The experimental design by Frantzis and Gremare (1992) was adopted to prevent contamination of the other treatments by the oily residue released by the salmon feed. Three dietary treatments were studied, including an extruded salmon feed (TROUW Supreme 6 mm Pellet); *Laminaria saccharina*, a macroalgae upon which "adult" *P. miliaris* are commonly found feeding in the wild (Bedford and Moore 1985); and an "unfed" (control). The horizontal test diameter of each animal was measured to the nearest 0.05 mm using the dial callipers at the beginning of the experiment and at monthly intervals thereafter. The trial began in February 1997 and lasted for 5 months.

"Adult" (Test Diameter: 21.3 mm to 21.7 mm)

Sea urchins were treated as described for the "intermediate" size class. This trial began in July 1997 and lasted for 6 months.

Gonadal Growth

One hundred and fifty, "adult" sea urchins (test diameter, 21.8 mm to 23.5 mm) were placed into each of nine, 250 L circular, black tanks supplied by a continuous flow through system with a flow rate of 1500 ml min⁻¹. The groups were either fed on *L. saccharina* or Salmon Feed (TROUW Supreme 6mm Pellets), and the control group had no additional feed. The trial began in February 1996 and was conducted for 12 months.

Gonadal growth was calculated on a monthly basis using the gonad index (GI), using the formula adapted from Gonor (1973):

$$GI (\%) = \frac{\text{wet weight gonads (g)}}{\text{wet weight eviscerated test (g)}} \times 100$$

The GI was determined for 10 individuals, selected at random from the population collected in Loch Creran at the start of the experiment. Thereafter, the GI of five randomly selected urchins from each replicated treatment group was calculated on a monthly basis. Both the wet weight of the eviscerated test and the gonadal wet weight were measured to the nearest 0.005 g after air drying for 2 min. In addition, the horizontal test diameter was measured

to the nearest 0.05 mm at monthly intervals, and the number of sea urchins that spawned upon dissection was recorded.

Statistical Analysis

The data were tested for normality (Shapiro-Wilk 1965) and variance homogeneity (Bartlett's Test; Zar 1996). Gonadal growth data were transformed using the ARCSINE transformation to produce a normally distributed dataset. The difference between the test diameter, the gonad index (%), and the total number of urchins at the start and end of the experimental trials was used to assess somatic, gonadal growth, and mortality rates, respectively. Analysis of variance (ANOVA) with the TUKEY multiple comparison test (Zar 1996) (MINITAB, Release 9 for Windows) were used to assess these differences between the three dietary treatments for each size class.

RESULTS

Somatic Growth

Juvenile

The hatchery-reared sea urchins fed salmon feed and the macroalgae, *Ulva lactuca*, significantly increased their test diameter (Fig. 1a) over the 6-month experimental period compared to the control group (TUKEY, $p < .05$). There was no significant difference in the final test diameter between the sea urchins receiving food, regardless of the diet type, and there was no significant growth observed in the control group by the end of the experiment (Table 1). A significant difference in mortality rates was observed between the three groups (one way ANOVA, $p = .022$) with the highest rates being found in the control group, although a small number did survive the 6 months without additional food. Lower mortality rates were observed in the urchins fed on *U. lactuca* (18.7%) and salmon feed (30.7%), although the only significant difference was between the urchins fed the macroalgae and the control group (45.3%) (TUKEY, $p < .05$). In appearance, it was observed that the sea urchins fed on the macroalgae had noticeably longer spines and a more flattened test as compared to the urchins fed salmon feed. The sea urchins fed salmon feed also had a redder test and gut wall coloration than the urchins fed the macroalgae.

Intermediate

In the intermediate size class, a significant difference (one way ANOVA, $p < .001$) in the growth rate was observed between the treatment groups over the experimental period (Table 1). Sea urchins fed salmon feed grew to a significantly larger size than the other two treatment groups (Fig. 1b). There was no significant difference between the growth rates of the sea urchins fed the macroalgae and the unfed sea urchins (TUKEY, $p < .05$). There was no significant difference in mortality rates between the three classes, although the highest mortalities were still recorded for the control group (14.7%). *P. miliaris* fed salmon feed were observed to be of a similar coloration to the sea urchins fed on the same diet in the "juvenile" growth trial.

Adult

In the adult size class, a significant difference in somatic growth rate was observed between the three treatment groups (one way ANOVA; $p < .001$; Table 1). Salmon feed significantly increased the growth rate as compared to the other two treatment

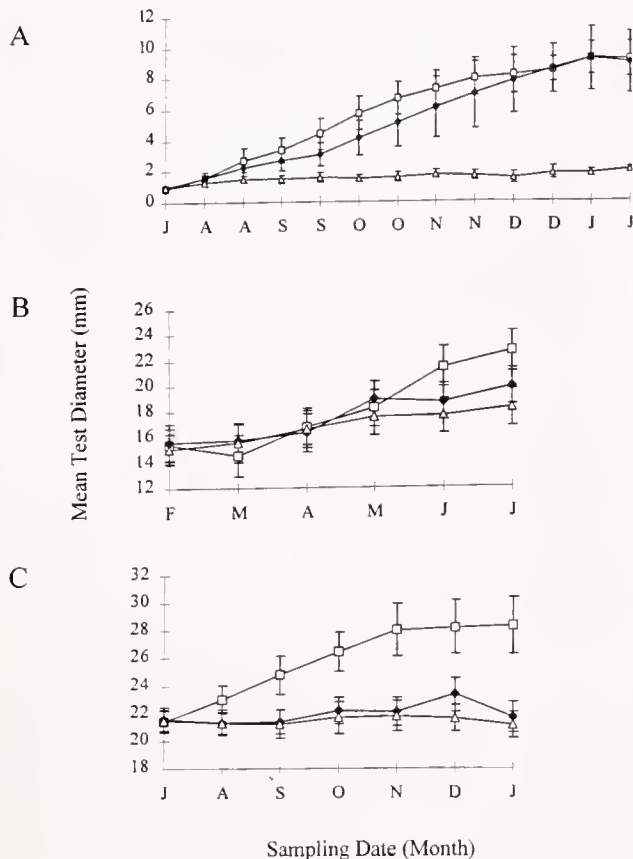


Figure 1. Somatic growth in three size classes of *P. miliaris*; (A) juvenile; (B) intermediate; and (C) adult. Mean values of test diameter (mm) and standard deviations (error bars) are shown for urchins maintained on either artificial diet (salmon feed) —□—; macroalgal diet —◆—; or unfed (control) —△—.

groups (Fig. 1c). No significant difference was found in the growth rate between the unfed sea urchins and those fed the macroalgal diet (TUKEY; $p < .05$). The urchins fed salmon feed were observed to have a much redder test and gut wall coloration and a darker peristomal membrane as compared to the other treatment groups. The control group were found to have a significantly higher mortality rate (54.7%) as compared to the urchins fed salmon feed (30.7%) and on the macroalgal diet (18.7%) (TUKEY, $p < .05$). There was no difference in mortality rates between the urchins fed salmon feed and the macroalgal diet.

Gonadal Growth

The GI and the annual reproductive cycle were significantly affected by the diet type. Sea urchins (test diameter: 21.8 mm to 23.5 mm) fed salmon feed showed a significant increase (TUKEY, $p < .05$) in gonad index (mean GI 1.84% to 57.19%) over the 12-month experimental period as compared to the maximum GI observed in June/July for the urchins fed the macroalgal diet (max. GI 12.0%) and the control (max. GI 4.9%) (Fig. 2a). The GI of the urchins fed the salmon feed increased rapidly in the first 8 weeks of the trial; the rate then slowed but continued to increase steadily for the remainder of the experiment. The GI did not show any sign of a reduction in mass after the typical spawning period (July to Sept), and the spawning period was considerably extended (Fig. 3). The sea urchins fed the macroalgal diet, however, followed the

typical gametogenic cycle observed in wild populations (Kelly et al. 1998), with a gradual increase in the GI from March to June, followed by a postspawning decline in June/July. The urchins that were unfed showed very little gonadal growth, with the GI remaining below 2%, although spawning was observed in a few individuals in June (Fig. 3).

A significant difference in the somatic growth rate was observed between all three of the treatment groups (one way ANOVA; $p < .001$; Table 1). Salmon feed was found to increase the growth rate significantly compared to the other two treatments over the 12-month trial period (Fig. 2b).

Diet also affected the color of the roe, with the sea urchins fed salmon feed producing gonads that were paler in color than those of the urchins fed on *L. saccharina*. However, the salmon feed did produce roe of a more consistent coloration than the algal diet. The unfed sea urchins only contained very small, dark brown gonads.

There was no significant difference in the mortality rates between the three treatment groups (one way ANOVA, $p = .056$), although the unfed group did have the highest mortality rate (35.1%) as compared to the urchins fed on the salmon feed (18%) and the macroalgal diet (18.9%).

DISCUSSION

This study shows that both somatic and gonadal growth in *P. miliaris* is influenced by diet type. The commercially manufactured salmon feed supported an increased somatic growth rate as compared to the macroalgal diet in the two largest size classes (i.e., test diameter ≥ 15 mm), concurring with the results of studies undertaken on other species of adult sea urchin, comparing artificial and natural diets (Nestler and Harris 1994, Fernandez and Bourdoursque 1998). In the juvenile urchins, however, there was no significant difference in somatic growth rates between the dietary treatments, and this result has also been observed in juvenile *S. droebachiensis* (<10 mm test diameter) fed an artificial, vegetable-based diet compared with the macroalgae, *L. saccharina* coated in the bryozoan *Membranipora membranacea* (Williams and Harris 1998). This apparent difference in dietary requirements between size/age classes in *P. miliaris* may be attributed to a change in resource partitioning, with nutrients being primarily directed to somatic production until the echinoid reaches a suitable size for gamete production, upon which the nutrients are then re-directed to gonadal growth, as suggested by Cellario and Fenaux (1990) and McShane and Anderson (1997). Jensen (1969) observed that *P. miliaris* were between 8 to 10 mm in test diameter before they contained gonads and were sexually mature. The data suggest that in the first few months after settlement, the macroalgal diet is as effective as the artificial diet and is able to provide sufficient quantities and/or the necessary nutrients when production is predominantly somatic. As the urchins increased in size/age (15 to 23 mm test diameter), however, the urchins fed the artificial diet outperformed the urchins fed the macroalgal diet, and the results suggest that the artificial diet is able to support both somatic and gonadal growth simultaneously in *P. miliaris*, unlike the majority of diets that have been prepared for other species of echinoid (Klinger et al. 1998).

The protein content of a diet has been identified as a major factor affecting production in echinoids (Frantzis and Gremare 1992). Salmon feed contains a significantly higher proportion of protein as compared to *L. saccharina* and *U. lactuca* (Table 2). The fact that there is a large difference in total protein and in the

TABLE 1.

Mean values ($n = 25$) and standard errors (in parentheses) of test diameters (TD) at the start and end of the experiments for groups of urchins maintained on either an artificial salmon feed, a natural macroalgal diet or unfed (control). Mean growth rates (mm/month) are also shown.

Size Class ($n = 25$)	Time (Mon)	Diet Type	TD (mm) (Start)	TD (mm) (End)	Mean Growth Rate (mm/month)
Juvenile	6	Salmon feed	0.91 (0.03)	9.21 (1.03) ^a	1.38
		Macroalgae	0.96 (0.02)	9.22 (2.06) ^a	1.38
		Control	0.99 (0.02)	2.0 (0.14) ^b	0.17
Intermediate	5	Salmon feed	15.45 (1.28)	22.7 (1.56) ^a	1.45
		Macroalgae	15.65 (1.40)	19.91 (1.41) ^b	0.85
		Control	15.10 (1.22)	18.26 (1.48) ^b	0.63
Adult	6	Salmon feed	21.42 (0.79)	28.43 (2.05) ^a	1.17
		Macroalgae	21.61 (0.81)	21.72 (1.22) ^b	0.02
		Control	21.52 (0.80)	21.18 (0.95) ^b	-0.06
Adult (GI trial)	12	Salmon feed	21.92 (0.53)	32.17 (0.66) ^a	0.85
		Macroalgae	21.96 (0.66)	24.67 (0.53) ^b	0.23
		Control	23.25 (0.38)	22.02 (0.27) ^c	-0.62

^a Means followed by the same letter for each size class are not significantly different (ANOVA; TUKEY).

protein base between the salmon feed and the macroalgal diet, may account for the significant difference in somatic growth rates observed in *P. miliaris*. Unfortunately, the exact protein requirements for somatic growth in *P. miliaris*, or any other species of echinoid, remain to be elucidated. Therefore, further nutritional studies are required to determine whether the quantity of total protein or the proportion of constituent amino acids are important in influencing the rate of somatic growth.

The salmon feed also caused a significant increase in the gonad index as compared with the macroalgal diet. The increase was comparable to the growth rates observed by Kelly et al. (1998) in *P. miliaris* maintained in cages alongside Atlantic salmon. The current study suggests that the sea urchins in the salmon cages may have obtained a high proportion of their diet from uneaten salmon pellets rather than from any other dietary source (e.g., net fouling). Somatic growth was also measured in this trial and was observed to follow the same general pattern as the growth measured in the largest size class (i.e., adults), confirming that the salmon feed was able to promote both somatic and gonadal growth simultaneously.

Echinoid gonads are a site for nutrient storage, as well as gamete production (Fernandez et al. 1995), with their major nutrient

components consisting of polysaccharides, proteins, and lipids. Biochemical analysis of the gonads of wild *P. miliaris* found that they were composed of 3 to 29% carbohydrate, 40 to 70% protein, and 10 to 17% lipid, depending on the reproductive state (Comely 1979). In the initial stages of this trial, there was a rapid increase in the gonad index of the sea urchins fed the salmon feed, which may have been caused by a proliferation in the storage cells (i.e., nutritive phagocytes) (Bryne 1990) and caused by the ingestion and efficient absorption of a diet rich in both protein and lipid (Table 2). The urchins fed the macroalgal diet, however, showed substantially slower gonadal growth, suggesting that their nutritional requirements were being met at a slower rate than that provided by the salmon feed, causing the increase in GI to occur at a reduced pace and over a more prolonged period of time. A study on *P. lividus* suggested that diets rich in proteins should induce high gonad production (Fernandez et al. 1995), although again the precise nutritional requirements for gonadal growth in echinoids is still unknown.

Sea urchins in this trial spawned at the same time of year, regardless of the diet, suggesting that temperature and/or photoperiod may be the environmental cue for gamete release in *P.*

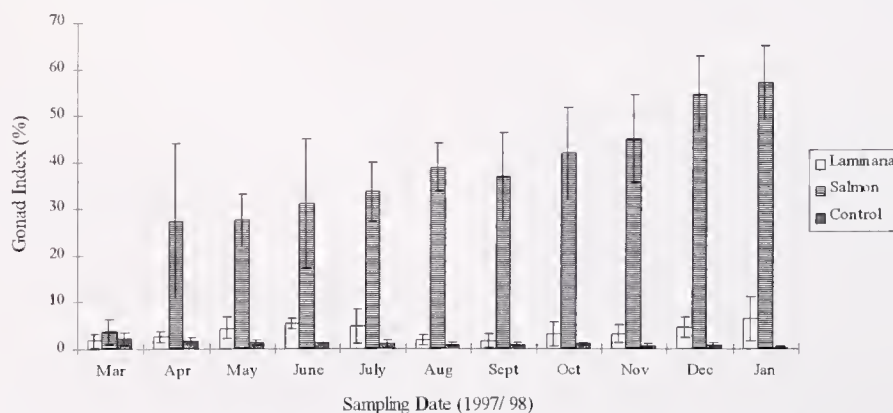


Figure 2a. Gonadal growth in *P. miliaris* ($n = 15$) fed either a commercially manufactured diet (Salmon Feed), a macroalgal diet or unfed (control). Mean GI (%) and standard deviations (error bars) are shown.

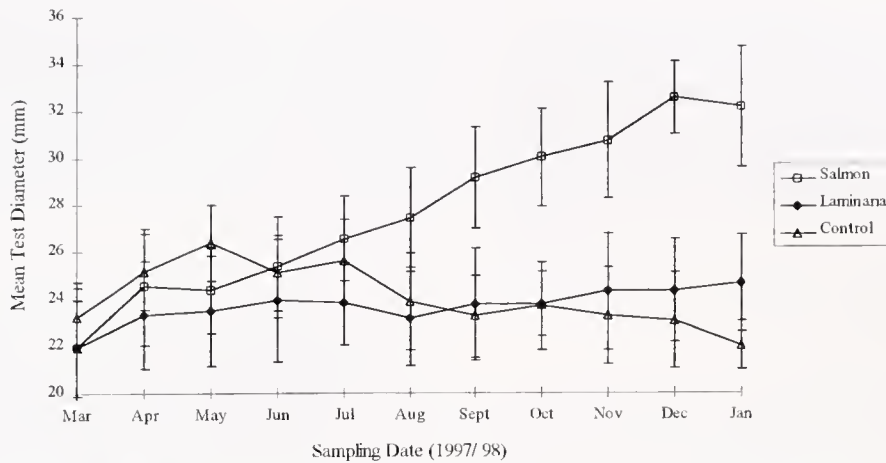


Figure 2b. Somatic growth of *P. miliaris* ($n = 15$) in the 12 month GI trial, fed either a commercially manufactured diet (salmon feed), a macroalgal diet or unfed (control). Mean test diameter (mm) and standard deviations (error bars) are shown.

miliaris (Elmhirst 1922, Jensen 1969). The spawning period in the sea urchins fed salmon feed, however, was considerably extended, and the postspawning decline in the GI was negligible as compared to the urchins fed the macroalgal diet. Similar results have been observed in *P. lividus* fed on an artificial diet (Fernandez et al. 1995). An inverse relationship between the quantity of nutritive phagocytes and gametes has been observed (Bryne 1990), with a transfer of nutrients between the two cell types. The absence of a postspawning decline in the sea urchins fed salmon feed, therefore, suggests that this transfer of nutrients from the nutritive phagocytes to the gametes can take place continuously, as long as there is an adequate input of the correct nutrients. In the case of the sea urchins fed macroalgae, the main period of spawning lasted for approximately 8 weeks and was followed by a substantial decline in the GI. *L. saccharina* contains much less lipid and protein than that found in the salmon feed, although the carbohydrate content is higher in the macroalgae as compared to the salmon feed (Table 2). Echinoids are able to digest both soluble and structural carbohydrate efficiently (Frantzis and Gremare 1992), and a role of their gut bacteria in promoting this process has been postulated (Fong and Mann 1980). The fact that gonadal growth, however, was relatively slow in the urchins fed on macroalgae and that they were only able to produce gametes for a relatively short period of time suggests that a high level of carbohydrate in the diet is not as

important as a high protein and lipid content in influencing gonadal growth and gametogenesis. The gonad index used here, provides information on gonad mass and not the relative proportions of the two main cell types. A histological study is, therefore, required to determine how artificial compared to natural diets affects the production rates of nutritive phagocytes and gametes.

The effect of diet type on spine length has also been observed in *S. droebachiensis* (Williams and Harris 1998), and it is suggested that the shorter spine length of the sea urchins fed salmon feed could be attributable to deficiencies in the artificial diet. This would have implications in the sale of this species, particularly in France, where a "live" sea urchin with long, healthy spines is required. The use of salmon feed alone, however, would be impractical on a large-scale basis because of the expense of the diet. The culture of this species in the U.K. is more likely to occur in a polyculture system, where a more varied diet will be available.

Test, gut, and gonad color also varied with diet type. The reddish test and gut coloration of urchins fed salmon feed may have been attributable to the incorporation of the anthraxanthin and canthaxanthin pigments present in the salmon feed. The pale color of the gonads of the sea urchins fed the salmon feed, however, suggest that the gonadal tissue was unable to absorb these dietary pigments. The gonads of the sea urchins fed the macroalgal diet, were generally brighter in coloration before spawning than the

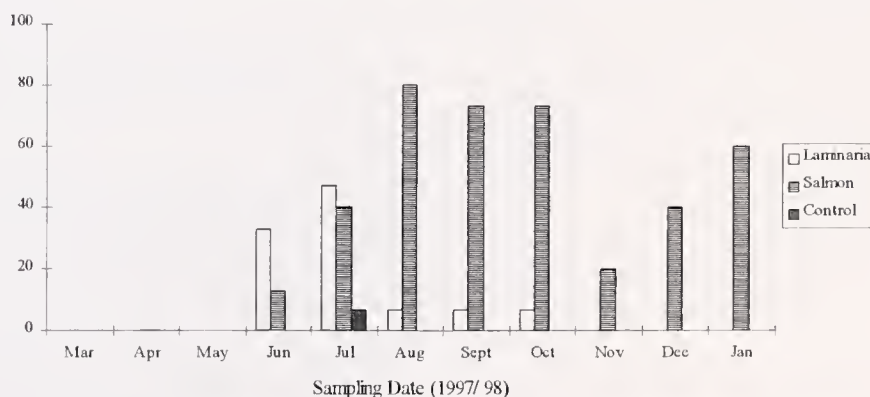


Figure 3. Percentage of *P. miliaris* ($n = 15$) spawning upon dissection when fed either a commercially manufactured diet (salmon feed), a macroalgal diet or unfed (control).

TABLE 2.
Composition of dietary treatments (% dry matter).

Dietary Constituents	Salmon Feed (Animal Based)	Macroalgae (Vegetable Based)
Protein	41% ^a	4–11% ^b
Lipid	31% ^a	4% ^b
Carbohydrate	7% ^a	23–27% ^c

^a TROUW Feed Analysis.

^b Bedford and Moore (1985), Black (1950).

^c Bedford and Moore (1985).

gonads of the sea urchins fed salmon feed, although there was much greater variation in the color. This may be a consequence of seasonal variations in the pigment concentrations found in *L. saccharina*.

To conclude, this study has found that *P. miliaris* is extremely versatile, because it is able to manipulate and ingest the salmon

feed pellets and to efficiently utilize a diet that is rich in both animal protein and lipid without any adverse effects on survival. The salmon feed significantly enhanced somatic and gonadal growth as compared to a natural macroalgal diet in *P. miliaris* with a test diameter ≥ 10 mm. It may be advantageous, therefore, to use the biochemical composition of salmon feed as a basis for the development of a more refined artificial diet for this potential aquaculture species.

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SOMATIC AND GONADAL GROWTH OF THE SEA URCHIN *PSAMMECHINUS MILIARIS* (GMELIN) MAINTAINED IN POLYCULTURE WITH THE ATLANTIC SALMON

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ABSTRACT Somatic (test) and gonad growth were recorded for the sea urchin *Psammechinus miliaris* (Gmelin) maintained in stocked salmon cages. Urchins of 11 to 16-mm test diameter maintained in pearl nets suspended in a stocked salmon cage showed a significantly greater increase in test diameter than urchins maintained in pearl nets away from the salmon farm. Urchins maintained in lantern nets suspended in stocked salmon cages showed enhanced gonad growth as compared with local field populations. The gonad growth was rapid over the winter months when in the field the urchins' roe content was typically low. Uneaten salmon feed pellets seemed to be an important part of the diet in the urchin/salmon polyculture system. The location and mesh size of their enclosures will affect the availability of uneaten salmon feed pellets to the urchins.

KEY WORDS: Sea urchin, polyculture, *Psammechinus miliaris*, salmon, gonad growth

INTRODUCTION

The sea urchin *Psammechinus miliaris* is found in localized, dense populations in sheltered sea lochs on the west coast of Scotland. The gonad of this species is considered to be of a good quality and, although smaller, fetches a higher price in the Parisian market than the more commonly eaten *Paracentrotus lividus* (Leighton 1995). In Scotland, field populations of *P. miliaris* normally have a low gonad content (Kelly unpub. obs.) However, the results of polyculture and tank-based trials (Kelly et al. 1998, Cook et al. 1998) have shown that feeding an artificial diet of commercially available salmon feed can produce substantial gonad growth in this species.

In a previous polyculture trial (Kelly et al. 1998) urchins free in cages containing around 10,000 two sea-winter salmon, produced a larger gonad than urchins free in empty salmon cages and fed *Laminaria saccharina*, the kelp on which they are commonly found feeding in the field. There were, however, difficulties in handling the two species simultaneously, and it was apparent that the urchins should be confined to separate enclosures within or adjacent to the salmon cages. Therefore, it became necessary to ascertain if urchins denied access to the organic debris on the cage floor would demonstrate the same rapid gonad growth. It was also unclear what the urchins were feeding on in the polyculture system, whether it was cage fouling, salmon food, salmon feces, or a combination of some or all of these. Hence, the term "in proximity to salmon" diet is used to refer to the source of food for urchins caged with, or adjacent to, salmon.

Because dietary requirements may vary with stage in the echinoid life history (Bedford and Moore 1985), a diet maximizing gonad growth in adults may have a different specification to one promoting somatic growth in juvenile urchins. The experiments described here were designed to show if the diet of urchins in proximity to salmon promoted somatic (test) growth as well as gonad growth and if the urchins' location within the salmon cage determined food availability and, hence, growth. It was intended that the results would allow deductions to be made about the relative importance of likely food sources in the polyculture system. In addition, the impact of maintaining urchins in proximity to

salmon on the urchins' annual reproductive cycle was studied. This information will aid in the design of the optimum polyculture system for these two species.

MATERIALS AND METHODS

"Proximity to Salmon" Diet and Somatic and Gonadal Growth

Urchins were collected from a field population exposed at low spring tides in Loch Glencoul, Sutherland, Scotland (N57°14'39", W 4°59'45"). Urchins of between 11 to 16-mm test diameter were selected, because this size range was considered to represent the size at which hatchery-reared juveniles could be transferred to sea. These urchins were used to stock three pyramidal "pearl" nets, (mesh size 5.0 mm) commonly used in the shellfish industry. The pearl nets were then moored in a sheltered bay in Loch Glencoul and away from any fish-farming activity. A further three pearl nets were stocked with urchins and suspended in a 15 m × 15 m × 13 m cage containing approximately 10,000 one sea-winter salmon in Loch Laxford, (N58°23'47", W 5°06'15"). Each net was stocked with 40 urchins, a density equivalent to 130 m⁻² of net surface area. The kelp was replenished in the nets at the control mooring when necessary; the urchins in the pearl nets in the salmon cage received no additional feeding. The horizontal test diameter of a subsample of 20 urchins from each net was measured at intervals over 7 months, from March 1997, using calipers adapted by the addition of metal pins designed to fit between the urchin spines. The urchins were measured on location and promptly returned to the same pearl net. A gonad index (GI) was calculated prior to, at one date during (July), and at the end of the experiment (October). The GI was calculated from a sample of nine individuals (three per pearl net) from each location and expressed as a percentage of the wet weight of the gonad divided by the wet weight of the eviscerated test. Gonad color was recorded upon dissection using a Sea Urchin Color Card (University of Maine and Maine Department of Marine Resources Fisheries Technology Service). Extrusion of gametes from either the dissected gonad or via the gonopores on dissection was recorded. The alimentary index (AI) was calculated at the end of the experiment as the dry weight of the alimentary

tract divided by the dry weight of the eviscerated test expressed as a percentage.

Effect of Location on Somatic and Gonadal Growth

P. miliaris were collected from Loch Glendhu (N58°15'49', W 4°57'45') by SCUBA divers. Forty urchins of 11 to 16-mm horizontal test diameter were placed in each of 12 pearl nets (5-mm mesh size). The experiment was conducted over 11 months from November 1996. The nets were situated at four locations, (1–4 below) in Loch Laxford, where the cages were stocked with approximately 10,000, one sea-winter salmon.

- (1) in the salmon cage, (3 m deep);
- (2) in the salmon cage, near the net cone, (8 m);
- (3) under the walkway, (3 m); and
- (4) under the walkway, (8 m).

At locations (3) and (4), the nets were suspended under the walkway that surrounds the salmon cage and were outside the salmon cage, 2 to 3 m from the cage wall. There were three replicate pearl nets at each location. The horizontal test diameter of 20 urchins was measured at intervals as described previously, and they were then promptly returned to the same pearl net. GIs were calculated from a sample of nine individuals (three per pearl net) from each location at the beginning, at one date during, and at the end of the experiment. The color of the gonad, the presence of mature gametes, and the AI were recorded as described above. The nets in shallower locations were brushed at intervals over the summer months to remove fouling organisms growing on the netting.

Effect of Cage Design on Gonad Growth

Urchins with a horizontal test diameter of >20 mm were collected by divers from Achriesgill Bay, Loch Inchard (N58°23'08', W 4°58'35'). Twenty urchins were placed in each of three pearl nets (mesh size 5 mm), giving a stocking density the equivalent of 66 m⁻² of net surface area. Their performance was compared with that of urchins in lantern nets (mesh size 12 mm), a cylindrical rather than pyramidal style of net, also commonly used in the shellfish industry. The nets were stocked with 40 urchins per compartment, an equivalent stocking density of 56 m⁻² of net surface area. Both net designs were suspended at location (2), as described above, on a raft of salmon cages at Badcall Bay (N58°18'08', W 5°09'52'), which were stocked with approximately 10,000, two sea-winter salmon. The GI of these urchins was recorded over 3 months from January 1997.

Influence of Proximity-to-Salmon on the Gonad Cycle

Urchins of test diameter >20 mm were collected by divers from Loch Glendhu and used to stock two replicate lantern nets (L1 and L2) (mesh size 12 mm) placed in a salmon cage in Loch Laxford stocked with approximately 10,000 one sea-winter salmon. Each of 10 compartments on the lantern nets were stocked with 20 urchins, equivalent of a stocking density of 28 m⁻² of net surface area, and the lantern net was suspended so that the top compartment was 8-m deep. The GI for 10 urchins from one compartment in each net was calculated at intervals over 1 year. Each compartment was only sampled once. The GI of these urchins was compared to that of a local field population that were accessible at low spring tides in Loch Glencoul. Here urchins were collected from two adjacent shore locations; W1, a sheltered bay typified by very fine sediments, surrounded by boulder cliffs, with few *L. saccharina* present and W2, which represented a more typical habitat,

boulder shoreline with a covering of *L. saccharina*. The urchins were collected from the mud flat, exposed a low tides, at W1, an atypical habitat type for this species and from the *L. saccharina* around the boulders on the shore line at W2.

After collection, the urchins were transferred promptly to the laboratory in insulated cool bins and maintained in a seawater aquarium until they were dissected (usually within 24 h). Test diameter, GI, AI, and gonad color were recorded as described above. The numbers of urchins extruding gametes on dissection was recorded. On three sample dates, one of the five gonads from three urchins from each site was preserved in Bouin's fluid for histological examination of the reproductive state. A central portion of the preserved gonads was dehydrated in alcohol, embedded in paraffin wax, and sectioned at 7 µm. The sections were stained with hematoxylin and eosin (H/E) and then assigned a reproductive stage from I to VI (Byrne 1990), describing the degree of maturity of the gametes present.

The data collected in experiments 1 to 4 were analyzed using nested analysis of variance (ANOVA) (Sokal and Rohlf 1995) and Student–Newman–Keuls (SNK) (Underwood 1997). The figures are provided with 95% confidence limits (CL). Means and standard errors of means (SE) are given in the Results section.

RESULTS

"Proximity to Salmon" Diet and Somatic and Gonadal Growth

The urchins used to stock the pearls nets had an initial mean test diameter of 13.3 mm (SE = 0.245, n = 40). At the end of the 7-month trial, the urchins in the salmon cage were significantly larger (Table 1) than the urchins at the control mooring that were fed *L. saccharina*. The mean survivorship of urchins from the pearl nets in both locations was high, 88%. The *L. saccharina* provided for the control group had a perforated appearance, indicative of urchin grazing.

The GI of the urchins in the salmon cage was significantly greater than that of the urchins at the control location on both sample dates (Table 1). Of the urchins sampled in July, three from the salmon cage and one from the control location extruded gametes from the dissected gonad, most appeared spent. By October, no animals extruded gametes upon dissection. The percentage of animals that had gonad of a good color in terms of its acceptability to the European markets (a pumpkin color) remained the same on each sample date, 78% in the salmon cage, 11% on the control mooring. At the end of the experiment, the AI of urchins in the salmon cage was also significantly greater than the AI of the urchins on the control mooring (Table 1).

Effect of Location on Somatic Growth

The average diameter of the urchins used to stock the pearl nets was 13.4 mm (SE = 0.341, n = 60). There was no significant difference in test diameter between the urchins in the four different locations after 7 months (June) on the salmon farm (Table 2). After this time, the pearl nets in location (2) were lost. There was a significant difference in the final test diameter of the urchins in the three remaining locations after 11 months (October). Urchins at 8-m deep under the walkway [location (4)] were significantly larger than those at both 3-m deep locations (SNK, p < .05). There was no significant difference between the two 3-m deep locations [(1) and (3)]. The over-all mean test diameter for urchins in the remaining three locations was 16.95 mm (SE = 0.121, n = 180).

TABLE 1.

Mean test diameter (mm), gonad index (GI), and alimentary index (AI) for urchins maintained away from the salmon farm and in salmon cages (standard errors in parenthesis).

Location	Diam. mm, Month 7, n = 60	GI, Month 4, n = 9	GI, Month 7, n = 9	AI, Month 7 n = 9
On control mooring	13.4 (0.214)	1.68 (0.321)	1.63 (0.887)	0.628 (0.256)
In salmon cage	17.6 (0.180)	11.02 (1.028)	11.59 (1.425)	2.35 (0.174)
Mean square	520.42	369.05	223.36	13.32
Degrees of freedom	1, 4	1, 4	1, 4	1, 4
F value	45.42	277.27	9.70	38.62
Significance	<.01	<.001	<.001	<.01

There was no significant difference between the GIs of urchins in locations (1), (3), and (4) after 8 months (July). However, there was significant difference in GI after 11 months (October), on this occasion the urchins under the walkway [(3) and (4)] had significantly higher GIs than the urchins in the cage (SNK, $p < .05$). There was no significant difference between urchins at locations (3) and (4).

Peak GIs were recorded in June (GI = 18.48). The gonad color of the urchins in the salmon cage was good in terms of its acceptability to the market place; 83 and 78% of urchins in June and July, respectively, were scored as having an acceptable gonad color. Observations recorded on the color of the alimentary tract showed that the urchins in locations (1) and (3) above (in the photic zone) had a normal gut color, a dark brown. The urchins in deeper locations had a gut color typical of that seen in urchins fed salmon food, orange and gray. There was no significant difference in AI for urchins in locations (1), (3), and (4) in month 11 (October). There was no quantitative recording of the extent of the fouling growing on the nets in different locations, but observation showed there was little fouling by marine algae on the pearl nets in deeper locations.

Effect of Cage Design on Gonad Growth

The GIs of the urchins in the pearl nets did not significantly increase from the initial value in January of 10.46 (SE = 1.775, n

= 10). After 3 months, the GI of the urchins in lantern nets at 8 m deep had significantly higher GI than urchins in the pearl nets (Table 3). The lantern net mesh was large enough to admit a whole salmon feed pellet, and urchins were observed feeding on pellets trapped in the mesh when the lantern nets were raised. The mesh of the pearl nets was too small to admit the feed pellets being fed to the salmon in these cages.

Influence of Proximity-to-Salmon Diet on Gonad Cycle

The GI of the urchins in lantern nets in the salmon cage increased from a mean of 4.39 (SE = 0.870, n = 10) to 19.93 (SE = 1.10, n = 20) (Fig. 1) in a 6-week period. This occurred in the winter months, which is out of the season for gonad growth in this species. The GIs showed an annual cycle, increasing until maximum values were obtained in May. The maximum mean GI recorded for the urchins in the salmon cage was 41.0, (n = 10) in May. The maximum GI recorded for an individual was 50.7, or 5.0 g of gonad. When the lantern nets were removed from the salmon cages for sampling, some urchins were observed feeding on salmon feed pellets that had become lodged in the mesh.

The maximum mean GIs for field populations were W1 33.0 (n = 10) and W2 17.8 (n = 10) recorded in June. The field population W1 (in the atypical habitat) had much higher GI's than W2,

TABLE 2.

Mean test diameter (mm), gonad index (GI), and alimentary index (AI) for urchins maintained in four different locations on the salmon farm (standard errors in parenthesis).

Location	Diam. mm Month 7 n = 60	Diam. mm Month 11 n = 60	GI Month 8 n = 9	GI Month 11 n = 9	AI, Month 11 n = 9
(1) Cage, 3m	15.07 ^a (0.273)	15.99 ^a (0.194)	11.69 ^a (1.093)	4.58 ^a (0.879)	0.724 ^a (0.168)
(2) Cage, 8m	14.34 ^a (0.224)				
(3) Walkway, 3m	14.46 ^a (0.153)	16.55 ^a (0.206)	11.84 ^a (0.897)	7.89 ^b (0.722)	1.56 ^a (0.216)
(4) Walkway, 8m	14.70 ^a (0.177)	18.19 ^b (0.179)	13.49 ^a (0.868)	8.71 ^b (0.840)	2.19 ^a (0.262)
Mean square	6.211	77.36	9.07	43.16	4.94
Degrees of freedom	3, 8	2, 4	2, 6	2, 6	2, 6
F value	1.60	5.56	1.83	6.03	3.45
Significance	NS	<.05	NS	<.05	NS

Values with the same superscript are not significantly different

TABLE 3.

Mean gonad index (GI) for urchins maintained in two different net types.

Net Design	Pearl Nets	Lantern Nets	Mean Square	Degrees of Freedom/ F Value	Significance
GI	10.35 (1.008)	19.08 (0.797)	342.47	1, 16 23.15	$p < .01$

Standard error in parenthesis, $n = 9$.

for this reason Figure 1 shows individual, rather than pooled, replicates. The high GI of urchins from W1, suggests they had a more abundant or more nutritious food supply. It was not readily apparent what this might be.

The postspawning regression of the gonad in the urchins in the salmon cage was reduced (Fig. 1), by August the GI for the field populations had fallen dramatically; whereas, the GI for urchins in the salmon cage remained high. The period over which ripe gametes were available was also extended in urchins from the salmon cage; 60% of the animals dissected were still shedding gametes in September, as compared with 15% of animals from the field population (Fig. 2a). Histological study of sectioned gonads confirmed that although the GIs of urchins in salmon cages were much higher over the winter months and early spring, the reproductive stage was similar to that of field populations. Urchins from both lantern nets and field populations had gonads representative of stage I to II (recovery and early growth phases) in December, stage IV in July (mature gametes present), and stage VI in September (spent). The increased mass of the gonad of the urchins in lantern nets was attributable to the increase in nutritive phagocytes, not to developing gametes.

The color of the gonads from dissected animals was variable, but desirable colors, in terms of marketability, were most prevalent from May to August (Fig. 2b). In the field population, there was a seasonal improvement in gonad color, higher percentages of animals with gonad of a marketable color being obtained from May to July when the GIs were also high. This also corresponded to the period when the most animals were in spawning condition. For the months when color was scored for both populations, the urchins in the salmon cages had a higher percentage of acceptable colors in 6 of the 8 months (Fig. 2b).

The AIs for the urchins in the salmon cages were much higher than those for the field populations (Fig. 3). There was much less variation between W1 and W2 in AIs than seen for GIs. The high AIs for the urchins in the salmon cage was reflected in the ap-

pearance of the alimentary tracts, which were large and fleshy, as compared with the field urchins'. There is evidence of an annual cycle in AI for both caged and field urchins, maximum values were obtained in April and May, and minimum values in September and August for salmon cage and field urchins, respectively. The annual cycle of AIs shows close correlation with the cycle of GIs. There was often a lack of fecal pellets or obvious semidigested material in the intestinal tracts of the urchins in the salmon cage.

The horizontal test diameter of the urchins removed from the lantern nets at each sample date increased significantly ($p < .005$) from a mean diameter of 24.07 mm to 32.25 mm ($n = 20$) during the course of the experiment.

DISCUSSION

The observed increase in test diameter (experiment 1) was the first indication that the salmon cage environment promoted somatic, not just gonadal, growth in urchins. This is an encouraging development in terms of the potential cost effectiveness of the polyculture system. The urchins increased in test diameter, even though they were suspended in the water column and were denied access to larger particulate debris of fish feces, uneaten pellets, and fish scales that accumulate on the cage floor.

No strong pattern emerged from the test growth and GI of urchins in pearl nets at different locations on the salmon farm (experiment 2). This, at first, would seem to suggest urchins could be maintained to equal advantage under the walkway, and hence minimize interference with the main crop, the salmon. However, the pearl nets used in these experiments had a mesh size that did not admit whole salmon feed pellets. Whatever food source the urchins benefited from in the absence of whole salmon pellets, it seemed to be equally available in the cage, under the walkway, and at the 3-m and 8-m deep locations. It is possible that the somatic growth rates in this polyculture system could be further enhanced if the urchins could have access to whole salmon feed pellets (Cook et al. 1998).

The different coloration of the gut from urchins in shallow and deep locations (experiment 2) suggests there were some differences in diet. The urchins in shallower locations may have eaten more fouling organisms from the pearl nets, because the growth of the fouling was limited at the deeper locations. However, because their GI and test growth were not significantly greater than those of urchins in deeper locations, light-dependant fouling organisms are not an important requirement for rapid growth in this system. The alternative food sources would seem to be degrading feed pellets, salmon feces, or dissolved organic matter (DOM). There is no direct evidence the urchins are feeding on salmon feces, which

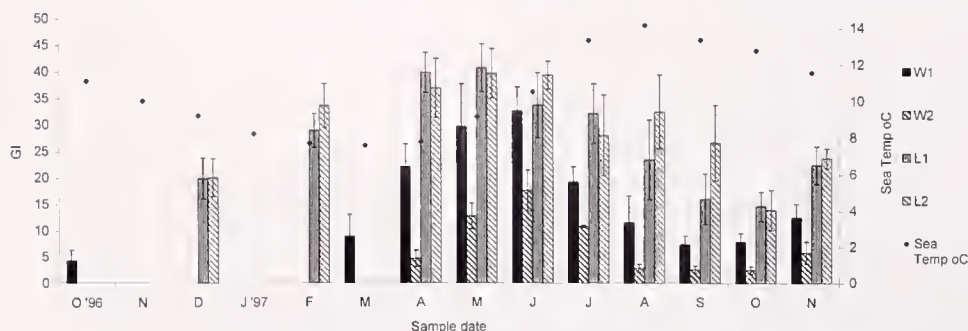


Figure 1. Comparative gonad indices (GI) of wild urchins (W1, W2) and urchins caged with salmon (L1, L2).

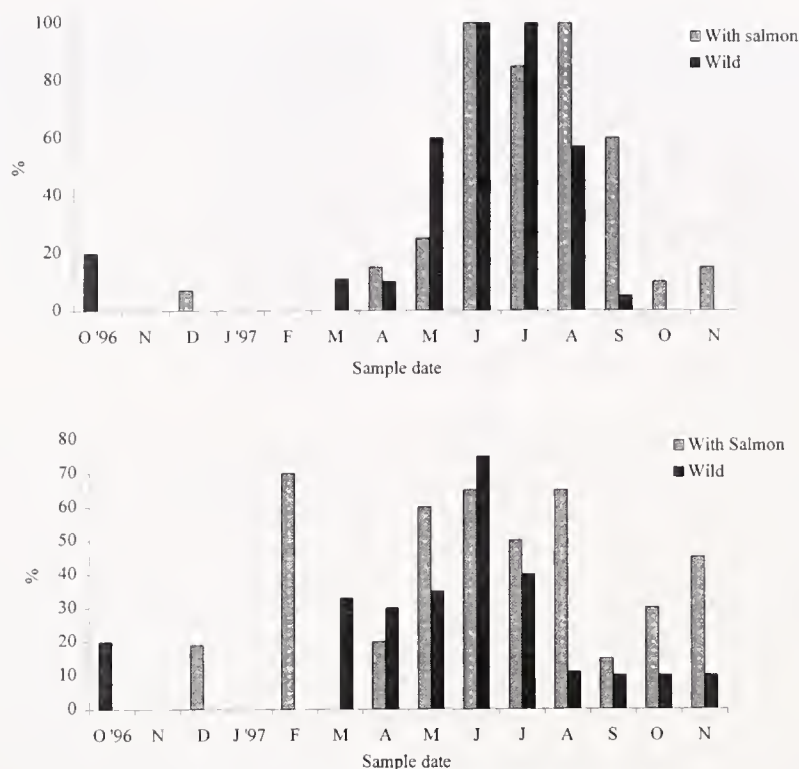


Figure 2a. Urchins extruding gametes at dissection (%).
Figure 2b. Gonad color acceptability (%).

are fairly liquid and do not appear to lodge in the urchin cage mesh. No attempt has been made to quantify the effect of DOM in this polyculture system; however, because urchins can uptake organic matter across the integument (Pequignat 1972, Pearse and Pearse 1973) it is possible that it contributes to the enhanced growth rates observed in these experiments.

Urchins maintained in lantern nets (experiment 3) attained significantly higher GIs than those in pearl nets in the same location. Because stocking densities were similar, the most obvious difference in the design of the two types of net was mesh size (5 mm in pearl nets and 12 mm in lantern nets). Urchins were observed feeding on salmon food pellets lodged in the lantern net mesh when the nets were pulled up for inspection. Lawrence et al. (1997) reported that *Loxechinus albus* was similarly able to manipulate and ingest extruded, pelleted feeds. It would seem that access to whole salmon feed pellets is vital to promote the rapid gonad growth seen in the urchins in lantern nets suspended in salmon cages.

This observation indicates it would be advantageous to site the urchins where they are most likely to encounter feed pellets missed by the fish. This factor should be taken into account in optimizing the cage design for urchins in polyculture with salmon. Good husbandry practice would dictate putting small hatchery-reared urchins to sea with smolts and then maintaining them with the same fish throughout their 2-year growth period to minimize the risk of disease transfer between fish cages. Keeping small urchins with young salmon that receive small pellets will facilitate adjusting the mesh size of the urchins' container to allow them to trap feed pellets more easily.

There is an obvious economic advantage to the salmon-farming industry of minimizing the amount of feed left uneaten by salmon;

however, the introduction of extreme efficiency in this respect may influence the productivity of urchins in this type of polyculture system.

The AIs support the observations made using GIs and test diameter that the salmon farm offers a rich feeding environment. The larger AI of urchins in proximity to salmon suggests they are using the alimentary tract to store additional nutrients. Histological examination of the gut from urchins on different dietary regimes could be used to support this observation. Annual cycles in gut size have been described in other species (Lawrence et al. 1965, Nichols et al. 1984). In *Echinus esculentus*, some degree of reciprocity has been recorded between GI and AI (Nichols et al. 1984), which is in contrast to the data presented here, which show a seasonal correlation between GI and AI in both field and caged populations. The decline in AI from April/May may represent the translocation of nutrients from gut to gonad for the production of maturing gametes during the spawning season.

The gonad color of the urchins in proximity to salmon was better in terms of market acceptability and also more uniform than

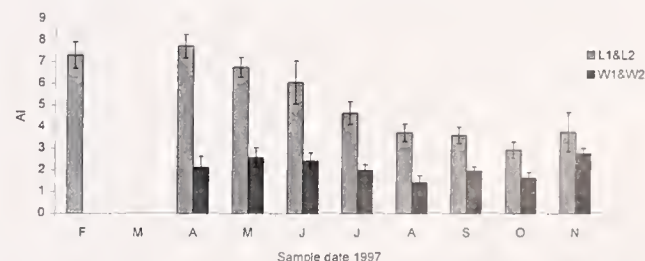


Figure 3. Comparative alimentary indices (AI) for wild urchins (W1, W2) and urchins in salmon cages (L1, L2).

that of the field urchins, reflecting their more standardized diet. In the field, *P. miliaris* are very opportunist feeders, an extensive list of food sources is given in Lawrence (1975). However, the pigments added to the salmon feed (astaxanthin and canthaxanthin) to promote a good flesh color did not particularly enhance the urchin gonad color. A bright orange coloration was observed in the gut of these urchins, perhaps indicating the pigments were expressed there. The ingestion and subsequent expression of dietary pigments in urchins is an area requiring further research.

Because the onset of gametogenesis in *P. miliaris* in proximity to salmon did not begin earlier than in the field population, the process is most probably triggered by a photoperiod or temperature or cue as in other echinoids (Pearse et al. 1986), rather than increased food availability.

In experiment 4, it would have been useful (and correct in terms of experimental design) to make a comparison between urchins in lantern nets in the salmon cage and urchins in lantern nets in cages empty of salmon but fed *L. saccharina*. However, market forces dictate that salmon growers can seldom afford to leave cages empty. Urchins were placed in lantern nets on a specially designed mooring away from the farm, but were repeatedly damaged by sea conditions. This serves to highlight the advantage of the polycul-

ture system, in that the use of existing aquaculture infrastructure eliminates the need to find additional sheltered sea sites and to fund purpose built urchin farms.

Although urchins that could not access whole salmon feed pellets showed significantly enhanced somatic and gonad growth in longer-term experiments, for rapid and sustained growth, the urchin cages should be designed with a mesh size large enough to admit a salmon feed pellet. They should be located where they are most likely to encounter salmon feed pellets missed by the fish. The aim of developing a polyculture system to produce market-sized urchins (40-mm test diameter) within a 2-year period is realistic.

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THE EFFECT OF PROTEIN CONCENTRATION IN PREPARED FEEDS ON GROWTH, FEEDING RATE, TOTAL ORGANIC ABSORPTION, AND GROSS ASSIMILATION EFFICIENCY OF THE SEA URCHIN *STRONGYLOCENTROTUS FRANCISCANUS*

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ABSTRACT Small *Strongylocentrotus franciscanus* (mean \pm SD = 18.3 \pm 13.1 g wet body weight) were placed in individual containers on 27 March 1997 and fed prepared feeds with 30, 40, and 50% protein until 30 January 1998 (n = eight/treatment). Growth, increase in wet body weight (including coelomic fluid) did not differ significantly with treatment [analysis of variance (ANOVA) p = .461]. The gross assimilation efficiency (increase in g dry body weight/g dry feed consumed) was calculated for each individual. The assimilation efficiencies for the 40 and 50% treatments did not differ significantly, but the efficiencies for the 30 and 40% treatments and the 30 and 50% treatments did differ significantly (p = .009). Total organic absorption was significantly different between the 50% and the other two diets treatments (p = .02) in July and January. Pooled male and female gonad protein and lipid concentration as a percentage of dry matter were significantly different, *t*-test, p = .002, p = .008, respectively. This suggests physiological regulation of feeding, with feeding rate decreasing with increased level of protein. The gonads of small *S. franciscanus* were in the premature stage of development at the end of the study.

KEY WORDS: *Strongylocentrotus franciscanus*, protein, absorption, assimilation, prepared diet

INTRODUCTION

Most attention on nutrition of sea urchins has involved large reproductive individuals. However, understanding the nutrition of small individuals is essential to minimize the time to marketable size. In general, somatic growth of small sea urchins on prepared diets is slow (Nagai and Keneko 1975, Lawrence et al. 1991, Pearse et al. 1995) in contrast to those fed single or mixed algal diets (Morris and Campbell 1996, Floreto et al. 1996, Thompson 1982). Understanding the response of small sea urchins to individual nutrients in prepared diets is one way to assess the importance of feed components to exploit the growth potential and achieve maximum return for the aquaculturist.

Growth in small sea urchins is primarily in the body wall (Lawrence and Lane 1982). Because protein is a major organic constituent of the body wall (Lawrence and Guille 1982, Lawrence and Byrne 1994, Fernandez 1997), supplementing prepared diets with protein may enhance somatic growth. Absorption of protein from prepared feeds is generally high, 60 to 80% (Lowe and Lawrence 1976, Klinger et al. 1994), indicating the possible importance of protein concentration in prepared feeds. Lower protein requirements would be desirable to reduce costs. In addition, because many current and proposed sea urchin aquaculture operations will occur in nearshore environments, reduction of protein concentration will decrease the problem of dealing with waste in maintaining water quality.

In California, the sea urchin *Strongylocentrotus franciscanus* (Agassiz) is a commercially and ecologically important species. It has supported a commercial fishery since the early 1970s (Kato

and Schroeter 1985) and continues to be among the most valuable of the states fisheries (Kalvass and Hendrix 1997). *Strongylocentrotus franciscanus* grows relatively slowly (Ebert and Russell 1993, Kato and Schroeter 1985) and exhibits variable growth (Botsford et al. 1994, Ebert and Russell 1992). The nutritional requirements for its growth are not known. Adequate interpretation of the nutritional quality of a feed requires knowledge, not only of the feed's composition, but the response of the animal in terms of feeding, absorption, and assimilation. In this paper, we evaluate these responses in small *S. franciscanus* to protein concentration.

METHODS

Small *Strongylocentrotus franciscanus* (n = 24), were collected from Fort Bragg, California (39°24'N, 123°48'W) in March 1997. They were transported to the laboratory in styrofoam coolers maintained at 11°C. After 7 days, the sea urchins were drained for 3 minutes on paper toweling, weighed to the nearest 0.01 g, and horizontal test diameters (HD) were measured at the widest point with vernier calipers to the nearest 0.1 mm. Initial body weight was 18.3 \pm 4.3 g and HD was 34.2 \pm 3.1 mm (mean \pm SE). We estimate this group of urchins was a 2-year-old cohort based on the settlement curves of Wing et al. (1995).

Individual urchins were placed in each test container of 18 l (10 \times 10 \times 25 cm) with seawater introduced from a common manifold at 0.67 \pm 0.1 l/min (mean \pm SE). Seawater was filtered to 500 μ in the flow through system at the Humboldt State University Marine Laboratory at Trinidad, California. Seawater temperature and salinity were recorded every 1 to 3 days. Eight test containers

per diet treatment were randomly distributed in an area of the laboratory receiving natural light. Sea urchins could move freely and were randomly distributed in their containers. Weight and HD were measured every 67 to 92 days to measure growth. Survival was 100% in all treatments.

Three agar based diets (5% meal, 5% agar, and 90% filtered seawater) were prepared weekly for 44 weeks, 27 March 1997 to 30 January 1998. Each individual was fed a $2 \times 2 \times 1$ cm piece of the prepared feed weighing 13 to 15 g every 3 to 4 days (8–9 feedings per month). The sea urchins moved directly to the food or were on top of it within a few hours of introducing the feed.

The fraction of food lost when immersed in seawater was determined by placing preweighed cubes of the agar-based diet in test containers without sea urchins for 4 days. The food was removed, patted dry with paper towels, and reweighed. The difference in weights before and after immersion was $0.003\text{g} \pm 0.001$, $0.007\text{g} \pm 0.004$, $0.009\text{g} \pm 0.004$ for the 30, 40, and 50% protein diets, respectively, (mean \pm SE, $n = 12$ for each diet). The test was done using a Sartorius balance accurate to 0.001 g. Food introduced and uneaten food removed during the experiment were weighed to 0.01g.

Uneaten feed was dried on paper toweling for 3 to 4 minutes before weighing to 0.01 g. For the first 3 months, the uneaten feed was dried at 60°C for 72 hours. A factor to convert wet feed weight to dry weight was calculated for each diet for the remaining 7 months. The percentage dry weight of the diets was $12\% \pm 0.3\%$ ($n = 424$). The dry weight of uneaten feed per feeding interval was used to calculate food consumption rate. Food consumption was calculated by subtracting the weight of uneaten food from that of the provided food. Food consumption rates were calculated per month for 10 months, April through January and for the intervals between weighing the sea urchins. Gross assimilation efficiency was calculated for the intervals between weighing the animals, as the increase in [(dry body weight (g) / dry feed consumed (g)) \times 100. For comparison, gross assimilation efficiency was also calculated for the increase in wet body weight/total wet feed consumed \times 100.

Total organic absorption was calculated indirectly by the method of Conover (1966) as: $U' = [(F' - E')/(1 - E') (F')] \times 100$, where U' is the absorption efficiency, F' and E' are the concentrations of organic matter in the food and feces. Food and feces samples were collected between 7 to 21 July and 1 to 11 January. Feces were collected on filter paper, transferred to preweighed crucibles, and dried at 60°C for 72 hours. The ash-free dry weights of the food and feces were determined by placing the samples in a muffle furnace at 500°C for 4 hours.

Sea urchins were dissected on 30 January 1998 to measure wet and dry weights of gonad, test, lantern, and gut. Dry weights were measured after drying the body compartments at 60°C for 72 hours. Wet body compartment indices were calculated according to Lasker and Giese (1954). Dry body compartment indices were calculated as the [(individual dry body compartment weight)/(the sum of the dry body compartments)] \times 100.

Official methods were used to measure the percentage dry matter of protein and lipid in the gonads (Helrich 1990). Protein was measured using a Leco Protein Analyzer. Lipid was determined by ether extraction. The protein and lipid analysis required drying the gonads at 100°C for 12 hours using a forced air dryer. The protein and lipid concentration as a percentage of dry matter in the gonads was compared between males and females in each diet treatment by t -test and for a comparison of all male and all female gonads.

Body weight and HD, dry food consumption, gross assimilation efficiency, total organic absorption, body indices, and protein and lipid concentration of male and female *S. franciscanus* gonads were compared between all three diets by Model 1 (fixed factor) analysis of variance (ANOVA). Significant differences were compared using the Student–Newman–Keuls method (SNK). In all cases, there was no heteroscedasticity (Cochran's C : maximum variance/sum of variances) and the data were normally distributed (Shapiro et al. 1968). A multiple regression analysis of mean daily feed intake per month for *S. franciscanus* fed the three prepared diets compared to mean monthly seawater temperatures was completed (Mendenhall and Sincich 1996).

The three prepared, semipurified diets differed in protein and carbohydrate concentration (Table 1). Krill, soybean, and casein were included in the diets to provide crude protein levels of 30, 40, and 50%. Three replicate feed samples of 20 mg were analyzed for amino acid concentration by performic acid oxidation for 48 hours at 4°C, liquid phase hydrolysis in 6 N HCl for 24 hours at 110°C, redissolving the sample in citrate buffer with α -guanidino-propionic acid as an internal standard, and injection of 50 μ l samples into the Beckman 6300 amino acid analyzer. This method recovers all amino acids present in their respective proportions. Histological analyses of *S. franciscanus* gonad sections were prepared to determine the reproductive stages (Unuma et al. 1996).

RESULTS

Mean wet body weight and horizontal test diameters of the sea urchins in each feed treatment did not differ significantly with feed type during the experiment (Fig. 1 and Table 2). The increase in

TABLE 1.
Composition and proximate analysis (percentage dry weight) of the three prepared feeds of different protein concentration fed small *Strongylocentrotus franciscanus*.

Ingredient		30% Protein	40% Protein	50% Protein
Kelp		26.9	26.9	26.8
Wheat starch		23.6	10.5	0.0
Krill		15.8	15.6	15.8
Soybean		9.9	17.3	22.1
Gluten		6.4	17.4	17.0
Cellulose		4.0	4.0	4.0
Casein		2.9	0.2	6.3
Gelatin		2.5	0.0	0.0
Phospholipid		2.0	2.0	2.0
Fish oil		1.6	1.5	1.4
Vitamin premix		1.5	1.5	1.5
Mineral premix		1.0	1.0	1.0
Cholesterol		1.0	1.0	1.0
Potassium phosphate		1.0	1.0	1.0
Vitamin C		0.1	0.1	0.1
Other (<0.1% each)				
Nutrient		30% Protein	40% Protein	50% Protein
Protein, crude		30.0	40.0	50.0
Carbohydrate		42.2	30.7	20.2
Fat, crude		7.0	7.0	7.0
Fiber, crude		6.7	6.6	6.7
Ash, total		8.4	8.7	9.0
Moisture		5.7	7.0	7.1

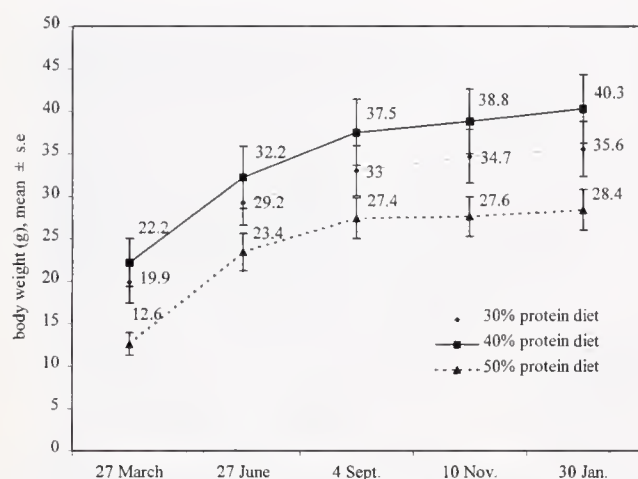


Figure 1. *Strongylocentrotus franciscanus*. Mean whole wet body weight through time for sea urchins fed three prepared diets.

wet body weight and HD over the experimental period was 15.5 ± 4.6 g and 5.76 ± 2.1 mm (mean \pm SE) for all sea urchins in the three diet treatments. Growth rate (increase in g wet-body weight/3 month and HD) decreased during the experiment. The indices of the body compartments of the sea urchins fed the three prepared diets did not differ significantly on either a dry or wet basis (Table 2).

Histological preparations showed all 24 *S. franciscanus* were in the premature stage of gametogenic development at the end of the experiment. Ovaries contained some mature ova in the center of the lumen. Previtellogenic and vitellogenic oocytes lined the follicle walls and were surrounded by nutritive phagocytes. The lumen of the testes were filled with some mature spermatozoa. Columns of spermatocytes are surrounded with nutritive phagocytes (Fig. 2). There were two females and six males in the 30 and 40% protein diet samples, and three females and five males in the 50% diet treatment.

The mean monthly dry food consumption rate differed significantly between diet treatments ($F = 5.6$, $p = .0126$, $df = 2, 29$) (Fig. 3). The SNK comparisons showed *S. franciscanus* fed 30 and 40% protein diets had significantly higher ($p < .05$) feeding rates

than those fed the 50% protein diet. Although not significantly different for any period, the rates of individuals fed feeds containing 30% protein were higher than those fed feeds containing 40% protein in all feeding periods. On one occasion, May, the feeding rates of *S. franciscanus* fed the 50% protein diet was greater than those fed the 40% protein diet. The trend of decreasing feeding rate with increasing concentration of protein in the feed can also be seen when the feeding rates are pooled for the four bimonthly periods when sea urchins were weighed and measured (Fig. 4).

Seawater temperature ranged between 12.5 and 16.8°C (Fig. 3). Temperatures in November and December were approximately 3 to 4°C higher than normal. Salinity was 31 to 33 ‰ for the 10-month experimental interval.

The multiple regression analysis of feed intake rate and seawater temperature showed no interaction between the treatments, as expected from the experimental design (Fig. 5). The slope of the three lines was similar, indicating the rate of increase of feed intake was the same for all three feed treatments. The temperature range measured during this study was within the normal range of temperatures for *S. franciscanus* in the natural environment and is reflected in the relatively low coefficient of determination (R^2) (Ulbricht and Pritchard 1972, McBride et al. 1997).

Total organic absorption efficiencies for *Strongylocentrotus franciscanus* were significantly higher for 30 and 40% protein feeds than for the 50% ($F = 4.97$, $df = 2, 20$, $p = .0191$; $F = 4.56$, $df = 2, 23$, $p = .0227$) for both July and January samples respectively (Table 3). The percentage of organic matter in the food and feces did not significantly change and was not significantly different for all diet treatments.

On a dry weight basis, gross assimilation efficiency was not significantly different between the 40 and 50% treatments, but the efficiencies for the 30 and 40%, and 30 and 50% treatments did differ significantly over the experimental period ($F = 5.878$, $df = 2, 23$, $p = .009$; SNK test $p < .05$) (Table 3, Fig. 6). Gross assimilation efficiency calculated for the four bimonthly intervals showed significant difference between the 30 and 50% efficiencies for the intervals 27 March to 27 June and 28 June to 5 September ($F = 6.161$, $df = 2, 23$, $p = .008$, $F = 4.224$, $df = 2, 23$, $p = .029$; all SNK test $p < .05$). Efficiencies of the 40 and 50% treatments were significantly different only between 27 March to 27 June, SNK test $p < .05$, on both a dry and wet weight basis.

TABLE 2.

Initial and final whole body weight and test diameter for *Strongylocentrotus franciscanus* fed three feeds of different protein concentration and final body indices on wet and dry weight basis ($n = 8$, mean \pm SE for all values).

Measurement		30% Protein Diet		40% Protein Diet		50% Protein Diet	
		Initial	Final	Initial	Final	Initial	Final
Whole animal weight (g)	Wet	19.9 \pm 4.9	36.6 \pm 6.5	22.2 \pm 5.6	40.3 \pm 7.6	12.6 \pm 2.6	28.4 \pm 4.7
	Dry	6.2 \pm 1.6	11.3 \pm 2.0	6.9 \pm 2.1	12.6 \pm 2.5	4.0 \pm 2.4	8.9 \pm 1.5
Horizontal test diameter (mm)		34.8 \pm 3.8	40.0 \pm 3.1	36.2 \pm 3.0	42.4 \pm 2.9	31.5 \pm 2.5	37.0 \pm 2.5
Test index (%)	Wet	54.8 \pm 0.08		54.8 \pm 1.3		55.8 \pm 0.7	
	Dry	80.2 \pm 0.9		80.9 \pm 0.9		82.8 \pm 0.8	
Gonad index (%)	Wet	16.7 \pm 0.7		15.9 \pm 1.2		15.4 \pm 1.1	
	Dry	11.6 \pm 1.0		10.8 \pm 1.0		9.3 \pm 0.9	
Lantern index (%)	Wet	3.9 \pm 0.1		3.9 \pm 0.2		3.8 \pm 0.2	
	Dry	6.1 \pm 0.2		7.7 \pm 1.5		5.8 \pm 0.2	
Gut index (%)	Wet	3.1 \pm 0.1		3.2 \pm 0.2		2.8 \pm 0.2	
	Dry	2.1 \pm 0.1		2.3 \pm 0.2		2.0 \pm 0.1	

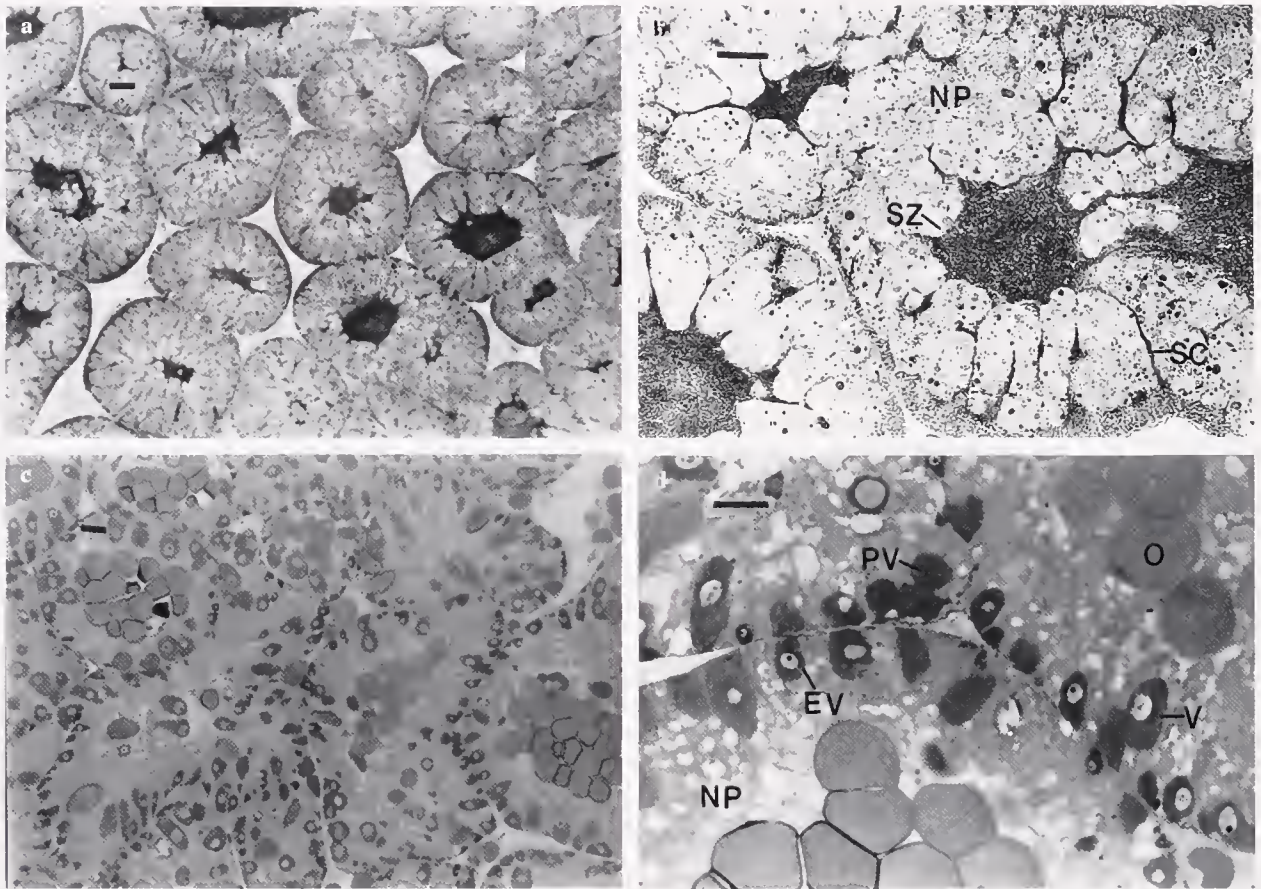


Figure 2 a,b. *Strongylocentrotus franciscanus*. Premature testes with primary spermatocytes (SC) and columns of spermatocytes project towards small masses of spermatozoa (SZ) in the central lumen. Nutritive phagocytes (NP) fill spaces around germ cells in the premature testes. Figure 2 c,d. *Strongylocentrotus franciscanus*. Premature ovary with some previtellogenic (PV), early vitellogenic (EV), detached vitellogenic (V), and mature ova (O). Nutritive phagocytes (NP) form a band around mature ova. Bar is 100 μ in all photographs.

Over the entire experimental period, and on a wet weight basis, gross assimilation efficiency was significant between the 30 and 50% ($F = 6.130$, $df = 2, 23$, $p = .008$; SNK test $p < .05$) (Table 3). The 30 and 50% efficiencies were also significantly different

between 27 March and 27 June ($F = 5.345$, $df = 2, 23$, $p = .013$, SNK test $p < .05$).

The gross assimilation efficiencies for the three levels of dietary protein decreased greatly from June to January, except for a

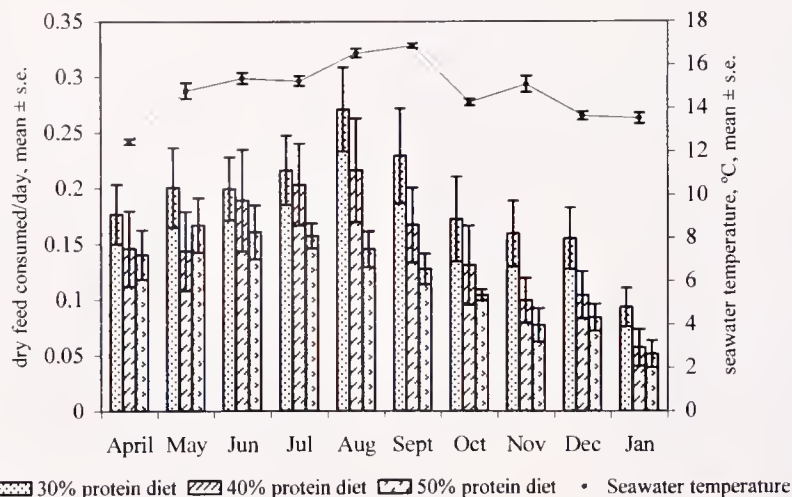


Figure 3. *Strongylocentrotus franciscanus*. Mean daily dry feed consumed per month for sea urchins fed prepared feeds of three different protein concentrations, $n = 7$ to 9 feedings per month or 19 to 25 seawater temperature records.

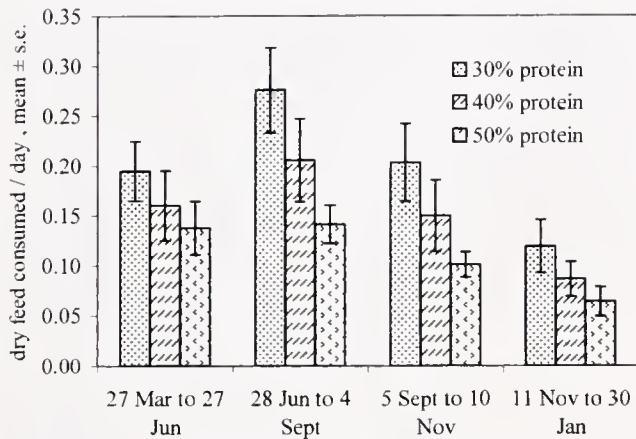


Figure 4. *Strongylocentrotus franciscanus*. Mean daily dry feed consumed for sea urchins fed three prepared diets for the time intervals between measurements of whole animal weight, $n = 8$ per treatment.

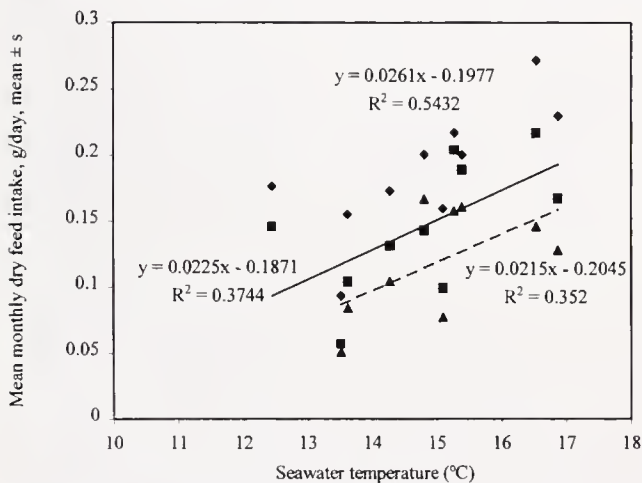


Figure 5. Regression lines for monthly mean seawater temperatures and mean daily food intake (g/day) for *Strongylocentrotus franciscanus* fed three prepared diets of different protein levels. ♦ 30% protein diet, ■ 40% protein diet, ▲ 50% protein diet; ----- linear (30% protein diet); — linear (40% protein diet); — — linear (50% protein diet).

slight increase for the 40 and 50% protein between the third time interval (5 September to 10 November) and the fourth time interval (11 November to 30 January), when dry weights were used.

Testes protein concentration showed no significant difference when sea urchins were fed feeds of three protein levels, $F = 2.34$, $df = 2, 16$, $p = .133$ (Table 4). Ovaries also showed no significant differences in gonad protein from the three feed levels, $F = 3.812$, $df = 2, 6$, $p = .118$. There were no significant differences in protein content of testes and ovaries of *S. franciscanus* in the 40% diet treatment, $t = -1.444$, $df = 2, 6$, $p = .296$. However, the 30 and 50% diet treatments showed significant differences between males and females, $t = -2.631$, $df = 6$, $p = .039$; $t = -5.743$, $df = 6$, $p = 0.001$, respectively. A t -test comparing pooled male versus pooled female gonad protein concentration was significant, $t = -3.522$, $df = 22$, $p = .002$.

Testes lipid concentration showed no significant difference when sea urchins were fed feeds of three protein levels, $F = 3.620$, $df = 2, 16$, $p = .054$. Ovaries also showed no significant differences in the three feed levels, $F = 3.314$, $df = 2, 6$, $p = .142$. There were significant differences in lipid content of testes and ovaries in the 30% diet treatment, $t = 3.942$, $df = 6$, $p = .008$. However, the 40 and 50% diet treatments showed no significant differences between testes and ovaries, $t = 0.906$, $df = 6$, $p = .400$; $t = 2.235$, $df = 6$, $p = .067$, respectively. Testes and ovaries from all diet treatments were pooled for a t -test, resulting in a significant difference in lipid concentration as a percentage of dry matter, $t = 2.919$, $df = 22$, $p = .008$.

The feeds contained 21 of the 40 different amino acids that can be detected by the Beckman 6300 analyzer (Table 5). Glutamic acid was the major amino acid, followed by glycine, aspartic acid, leucine, and proline.

DISCUSSION

The prepared diets with different protein concentrations fed small *Strongylocentrotus franciscanus* resulted in significant differences in feeding rate, gross assimilation, organic absorption, and gonadal protein and lipid concentration, but not in growth or gametogenic development. Somatic growth was greatest during the initial months, indicating the nutrients in the diets were available to the urchins and important for increased body size. The growth pattern of small *S. franciscanus* resembled the growth curves of

TABLE 3.

Strongylocentrotus franciscanus. Feed intake on wet and dry weight basis, total organic absorption, and gross assimilation efficiency for sea urchins fed three prepared feeds with different protein concentrations.

Measurement	n		Diet		
			30%	40%	50%
Daily feed consumption g/day (one per month per diet)	10	Wet	1.42 ± 0.07 ^a	1.25 ± 0.09 ^a	0.89 ± 0.06 ^b
		Dry	0.19 ± 0.02 ^a	0.15 ± 0.02 ^a	0.12 ± 0.02 ^b
% Organic matter in food (F')	24	July	71 ± 1 ^a	70 ± 1 ^a	65 ± 2 ^a
		Jan.	70 ± 1 ^a	69 ± 2 ^a	64 ± 4 ^a
% Organic matter in feces (E')	24	July	47 ± 2 ^a	47 ± 1 ^a	48 ± 3 ^a
		Jan.	49 ± 2 ^a	51 ± 2 ^a	51 ± 1 ^a
Total organic absorption (U') %	8	July	62 ± 3 ^a	62 ± 1 ^a	51 ± 4 ^b
		Jan.	57 ± 2 ^a	56 ± 2 ^a	46 ± 1 ^b
Gross assimilation, (27 March to 30 Jan.)%	8	Wet	4.3 ± 0.1 ^a	5.3 ± 0.1 ^{ab}	6.4 ± 0.1 ^b
		Dry	9.5 ± 0.8 ^a	14.6 ± 2.1 ^b	15.9 ± 0.8 ^b

Values in each row with the same letter are not significantly different ($p < .05$)

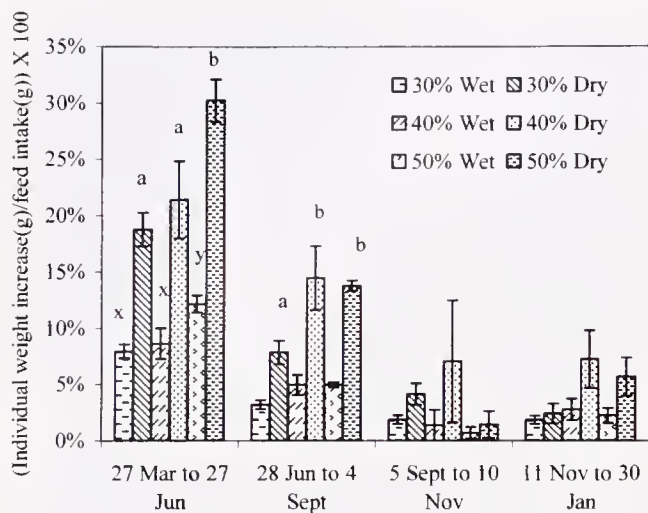


Figure 6. *Strongylocentrotus franciscanus*. Gross assimilation efficiency for sea urchins fed prepared diets of three protein levels. For each bar, $n = 8$, mean \pm SE. Within wet and dry gross assimilation values, bars with different letters are significantly different ($p < .05$), bars without letters are not significantly different.

Strongylocentrotus droebachiensis (Müller) fed less preferred feeds (Vadas 1977, Larson et al. 1980). The small *S. franciscanus* showed relatively high growth during the initial 3 to 4 months and reduced growth rates during the last 6 months. When fed *Nereocystis luetkeana* (Mertens) Postels and Ruprecht, *S. franciscanus* of 30-mm test diameter increased 50% over a 90-day period between May and August (Morris and Campbell 1996), similar to the initial growth pattern observed here. The opportunistic and selective feeding strategy (Vadas 1977, Andrew 1989) and the cyclical nature of gametogenic development of *S. franciscanus* (Bernard 1977) may also partially explain the higher initial growth followed by a reduced rate. Feeding rates reflected the reduced growth rates and are known to decrease in general with increased reproductive development (Lawrence 1975).

Over-all somatic growth of small *S. franciscanus* are similar to growth of other echinoids in other long-term studies (Fernandez and Caltigirone 1994, Thompson 1982). Although not statistically significant, the absolute growth of *S. franciscanus* fed a diet containing 40% protein was greatest of the three diets used. Tollini et al. (1997) found *S. droebachiensis* also had higher growth rates when fed a feed with 40% protein.

Gonadal production and reproductive development suggests the feeds supplied sufficient nutrition for reproductive growth. The small *S. franciscanus* may have developed gametes for the first time, because *S. franciscanus* become sexually mature at 50 mm

HD (Kato and Schroeter 1985). The timing of the reproductive cycle did not seem to be affected by the prepared diets. The study was terminated before the spawning season but included the gonadal growth season, indicating the gonads may have reached their maximum mass for small *S. franciscanus*.

Seasonal growth patterns correlated with reproductive development have been observed for other species of temperate echinoids. *Strongylocentrotus intermedius* (Agassiz) and *S. nudus* (Agassiz) cease somatic growth with gonadal development (Fuji 1967, Agatsuma et al. 1996). Slower growth of *Strongylocentrotus purpuratus* (Stimpson) results from gametogenesis prior to spawning (Pearse et al. 1986). *S. franciscanus* develops gonads seasonally (Bernard 1977, Pearse and Cameron 1991), but there are little direct data on seasonal somatic growth. Although the diets used here are quite different from natural algal diets *S. franciscanus* encounters in the nearshore environment, they indicate a seasonality of growth and feeding that may be similar in pattern but not in timing to other *Strongylocentrotus* spp. Adult *S. franciscanus* develop gametes in the late fall and early winter (Bennett and Giese 1955, Bernard 1977, McBride et al. 1997). If the small *S. franciscanus* in this experiment were experiencing the same timing of gametogenic development, the reduced feeding rates between September and January reflect reproductive development.

The unrestricted availability of the prepared diets during the 10-month study suggests a seasonal food consumption rate. Unlike algal preference studies, where feeding rates increased for the preferred foods, feeding rates with prepared diets of three protein concentrations were related to protein concentration and were highest on the lowest protein concentration. This suggests some adjustment by the urchins to the protein content of the diet. In starvation experiments, nitrogenous compounds in the complex coelomic fluid of *S. franciscanus* were maintained at minimum levels in experiments up to 5 months in length, also suggesting some physiological regulation of these compounds (Booltian and Giese 1959). The amino acid content of the three diets was similar, indicating the over-all protein content had more effect on the small urchins than the individual amino acids. However, the same amino acids found in the diets are found in unfertilized eggs of *S. purpuratus* (Kavanaugh 1953). In comparison studies using long- and short-chain amino acids as a protein source, arginine was the limiting amino acid for *Pseudocentrotus depressus* (Agassiz) in all prepared diets and algae tested (Akiyama et al. 1997).

Gross assimilation efficiency of *S. franciscanus* was greater for sea urchins fed the higher protein concentrations because of lower feed consumption rates. As a measure of food use, comparison of assimilation values between other studies are possible when dry weights are used (Parker 1987), and this has not usually been done for echinoids (Klinger 1998). Fewer significant differences and

TABLE 4.

Strongylocentrotus franciscanus. Protein and lipid concentration of gonads as a percentage of dry matter (mean \pm SE).

	Protein Level in Feed					
	30% Protein Diet		40% Protein Diet		50% Protein Diet	
	Male	Female	Male	Female	Male	Female
n	6	2	6	2	5	3
Protein	59.4 \pm 2.3	47.9 \pm 1.8	56.3 \pm 1.2	53.5 \pm 2.8	61.2 \pm 0.6	54.6 \pm 1.1
Lipid	21.3 \pm 0.8	27.7 \pm 0.9	24.2 \pm 0.9	26.0 \pm 2.2	22.1 \pm 0.5	23.7 \pm 0.3

TABLE 5.

Amino acid composition of three prepared diets containing 30, 40, or 50% crude protein expressed as a weight percentage ($n = 3$, mean \pm SD).

Amino Acid	30% Protein Diet	40% Protein Diet	50% Protein Diet
Alanine	4.9 \pm 0.08	4.1 \pm 0.02	4.0 \pm 0.06
Arginine	6.2 \pm 0.2	5.9 \pm 0.1	5.9 \pm 0.1
Aspartic acid	9.6 \pm 0.2	8.9 \pm 0.1	9.1 \pm 0.2
Cysteine	0.07 \pm 0.01	0.05 \pm 0.04	0.06 \pm 0.01
Cystine	0.2 \pm .01	0.6 \pm 0.2	0.4 \pm 0.01
Ethionine	0.2 \pm 0.02	0	0
Glutamic acid	21.2 \pm 0.3	26.2 \pm 0.3	25.1 \pm 0.3
Glycine	5.9 \pm 0.3	4.2 \pm 0.01	3.9 \pm 0.02
Histidine	1.6 \pm 0.1	1.6 \pm 0.1	1.8 \pm 0.01
Hypoline	.09 \pm 0.3	0	0
Isoleucine	4.5 \pm 0.1	4.5 \pm 0.02	4.5 \pm 0.03
Leucine	8.0 \pm 0.02	8.0 \pm 0.02	8.2 \pm 0.1
Lysine	6.2 \pm 0.2	5.8 \pm 0.2	5.6 \pm 0.1
Methionine	2.1 \pm 0.03	1.8 \pm 0.02	1.9 \pm 0.04
Phenylalanine	4.9 \pm 0.1	5.33 \pm 0.1	5.2 \pm 0.1
Proline	6.4 \pm 0.2	6.6 \pm 0.1	6.6 \pm 0.2
Serine	3.8 \pm 0.03	3.9 \pm 0.08	4.3 \pm 0.07
Taurine	0.3 \pm 0.02	0.2 \pm 0.01	0.2 \pm 0.02
Threonine	3.6 \pm 0.2	3.3 \pm 0.04	3.5 \pm 0.01
Tyrosine	3.6 \pm 0.1	3.8 \pm 0.004	4.1 \pm 0.03
Valine	5.5 \pm 0.1	5.3 \pm 0.01	5.5 \pm 0.01

trends in assimilation were shown when compared based on a wet weight basis, suggesting they are not as sensitive a measurement of growth as efficiencies based on a dry weight basis.

The multiple determinations of total organic absorption probably provide reliable estimates of the organic matter absorbed by small *S. franciscanus*. The quantity of organic matter absorbed by *S. franciscanus* was not significantly different in the summer and winter samples. This may reflect the consistent composition of the prepared diets. *S. intermedius* (Fuji 1967) had seasonal changes in total organic absorption. Seasonal variation in the algal diets may contribute to the fluctuations in absorption.

Echinoids fed preferred plant foods resulted in high absorption values (Vadas 1977, Lawrence 1975). Absorption values between 50 and 60% for urchins fed nonpreferred algal diets supported low growth and no gonadal development. Unlike studies with algal diets, the relatively low organic absorption of the prepared diets supported somatic and gonadal growth as well as gamete development. The 50 to 60% organic absorption values suggest the small *S. franciscanus* received the required nutrients for growth and body maintenance, utilizing a small amount of the food consumed. When fed agar based diets, *Paracentrotus lividus* (Lamarck) absorbed 12 to 30% of the organic material (Lawrence et al. 1989).

The protein concentration in the gonads of small *S. francisca-*

nus was higher than values for other temperate and tropical species collected from natural habitats or fed prepared diets and sampled at a season with similar gonad indices to *S. franciscanus* in this study (Geise et al. 1958, Geise 1961, Geise et al. 1964, Fernandez 1997). The protein concentration of *S. purpuratus* and *Allocentrotus fragilis* (Jackson) were approximately 30%; whereas, *Stomopneustes variolaris* (Agassiz) and *Paracentrotus lividus* were approximately 40%. The testes of *S. franciscanus* contained a higher protein content than ovaries, a relationship found in the other species. The protein concentration was considerably higher in the small *S. franciscanus*, 59 and 52% for testes and ovaries, respectively, than found in other studies, suggesting the protein in the diets was utilized by the sea urchins for gonad growth.

The lipids available to the small *S. franciscanus* in this study were close to those in natural diets of sea urchins (Lowe and Lawrence 1976, Floreto et al. 1996, McBride et al. 1997), and gonadal lipid concentration in the gonads was similar in all studies compared (Geise et al. 1958, Geise 1961, Geise et al. 1964, Fernandez 1997) to the small *S. franciscanus*. Gonadal lipid concentration was greater in ovaries than testes, a relationship found in other sea urchins that resulted from the higher lipid content of eggs than sperm (Geise 1961). The data from this study are not sufficient to determine if the protein and lipid levels are seasonal or not when sea urchins are fed prepared diets of equal nutritive value throughout the reproductive cycle (Bennett and Giese 1955, Bernard 1977).

Small *S. franciscanus* fed prepared diets of three protein concentrations increased test diameter and whole body weight, and showed significant differences in feeding rate, gross assimilation, and organic absorption. Food availability was unrestricted. Although we know little about what was biologically available, the urchins consumed, assimilated, and absorbed significantly different amounts of the three diets. Although not statistically significant, the sea urchins fed the 40% diet had the greatest over-all growth, a moderate feeding rate, a gross assimilation efficiency equivalent to those fed a 50% protein diet, and absorbed organic matter at the higher rate of 60%. For the aquaculturist, the 40% protein concentration in a diet seems to offer the greatest return and highest efficiency.

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EFFECT OF DIFFERENT FORMULATED DIETS AND REARING CONDITIONS ON GROWTH PARAMETERS IN THE SEA URCHIN *PARACENTROTUS LIVIDUS*

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ABSTRACT World annual production of sea urchins has increased regularly since 1980, and many countries, such as France, are currently confronted with the problem of overexploitation of stocks. For this reason, aquaculture of the sea urchin is a possible solution for the future. The study undertaken here aimed to determine physiological responses (growth) of *Paracentrotus lividus* given three different feed types varying in quality (vegetable or animal) and biochemical composition and under variable environmental conditions (temperature, light). Experiments in land-based tanks revealed that growth was greater when individuals were fed a mixed formulated feed containing both animal meal and plant meal or containing only animal meal, than when fed a feed containing only plant meal or a natural food, the seaweed *Cymodocea nodosa*. Maintaining a temperature of 20°C and rearing the sea urchins in the darkness did not have any noticeable effect on growth rate. The growth model established with data obtained from the tank studies for individuals fed the mixed feed indicated that the growth of the sea urchin can be maximized by appropriate feeding. Finally, the different treatments did not bring about any differences in the relative importance of the various body parts (gonads, gut, test, lantern) although the gonadal index in reared sea urchins was very high, even for small individuals (20–25 mm).

KEY WORDS: Sea urchin, aquaculture, growth, formulated feeds, *Paracentrotus lividus*

INTRODUCTION

The human consumption of a variety of a sea urchin species dates back to ancient times (Giot 1970, Le Direac'h 1987). In France, the species that is the most widely consumed is *Paracentrotus lividus* (Lamarck, 1816). The fisheries for this species is extensive and highly organized (Le Direac'h 1987). Since the collapse of the French sea urchin fisheries (Le Direac'h 1987, Le Gall and Bucaille 1987), the shortage has been met by increased Irish and Spanish imports (Biraïs and Le Gall 1986, Kelly et al. 1998). In light of this demand, and fueled by the high market value of the sea urchin roe, sea urchin farming has developed in France (Le Gall and Bucaille 1987, Fernandez et al. 1995, Fernandez 1996). The development of improved sea urchin rearing techniques requires finding an adapted feed and rearing system as well as a thorough understanding of the organism's growth processes.

In the present study, the technology chosen to develop sea urchin aquaculture is land-based rearing with the use of formulated diets. Although more expensive, such land-based systems are interesting, because they offer the possibility of controlling environmental conditions and may be the only approach possible in areas of limited access to sea-based resources. The advantages of using formulated feeds include the elimination of problems linked to food availability and nutritional content observed for natural food sources (marine plants), the limitation of environmental impacts associated with the utilization of these natural food sources, and the facilitation of storage and transport.

To date, most studies dealing with formulated diets have examined the effect of these diets on either gonad production (De Jong-Westman et al. 1995, Lawrence et al. 1997, McBride et al. 1997, Goebel and Barker 1998) or nutrition (for review see Klinger et al. 1998). Studies concerning the effect of formulated diets on growth are less numerous. Most of these examine sea urchin production as a function of different feed. The results generated generally reveal a greater growth with formulated diets than with their

natural counterparts (Lawrence et al. 1992, Fernandez and Caltagitone 1994, Williams and Harris 1998).

In addition to food quantity and quality, growth of echinoids depends upon several abiotic factors. Two of these are temperature and photoperiod (Kenner 1992, Guillou and Michel 1994, Pearse et al. 1995). In temperate waters, growth is seasonal and is characterized by an increase in growth during spring and summer (high temperature) and a decrease in growth in autumn and winter (low temperature) (Guillou and Michel 1994). For *Paracentrotus lividus*, the optimal temperature seems to be between 18 to 23°C (Le Gall et al. 1990, Grosjean et al. 1998). Photoperiod seems to influence partitioning between somatic and gonad growth (Pearse et al. 1986, Beyer et al. 1998).

The aim of the present study is to compare growth parameters in *Paracentrotus lividus* when this species is maintained under rearing conditions for long periods of time (6 months) and fed different types of formulated feed. The effect of various rearing conditions, such as light and temperature, is also examined.

MATERIALS AND METHODS

Collection of Echinoids

The experiments were conducted on the shore of the Urbinu lagoon (Corsica, Mediterranean). The site is characterized by a large population of *Paracentrotus lividus* (Fernandez and Caltagitone 1990), which can be used as a source of sea urchins for the farming scheme. For the purposes of this investigation, all sea urchins were collected from this lagoon, on pebbly bottoms (Fernandez and Boudouresque 1997), or produced in an experimental laboratory (for the smallest size class). Five size classes were used: 3 to 9 mm, 10 to 15 mm, 20 to 25 mm, 30 to 35 mm, and 40 to 45 mm (test diameter at the ambitus without the spines). After a 5-day acclimation period, the sea urchins were separated into groups (two replicates of 30 sea urchins per treatment except for the 3 to 9 mm size class, for which only one group was studied)

using plastic baskets with a mesh of 1 to 0.5 mm. The baskets were suspended 1 cm above the bottom of the tanks to prevent the sea urchins from reingesting their feces.

Experimental Set-up

The experimental set-up consisted of a rearing apparatus measuring 9 m × 4 m and housing three series of three superimposed tanks (3 m × 0.15 m × 0.5 m) (see Fig. 2). For each tank series, the force of gravity was used to circulate the water among the three tanks. The resulting cascade oxygenated the water. For two of these tank series, water flowed in an open circuit to the lagoon. The water turnover was 10 min. Experimental water temperature varied with the season (from 9 to 29°C, Fig. 1) and did not differ from lagoon water temperature. For the last tank series, the water flowed through a semiclosed circuit with a 50% new water turnover every 24 h. In this last tank series, the water temperature was maintained at 18 to 20°C year around.

Rearing Conditions

Sea urchin growth was studied as a function of three rearing parameters: (1) temperature (ambient or maintained at 18–20°C); (2) light (natural or darkness); and (3) feed (three formulated feeds and one natural food source). The natural food source was made up of living fronds of the seaweed *Cymodocea nodosa* (Ucria) Asherson sampled from the lagoon; whereas, the three formulated feeds were prepared (Table 1). The first feed consisted of vegetable meal and vegetable oils. This food is rich in soluble carbohydrates (58%) and was referred to as "vegetable feed." The second feed consisted of fish meal and vegetable meal in equal quantities mixed with fish oil and vegetable oil and was referred to as "mixed feed." Its biochemical composition is a mixture of soluble proteins (29%) and soluble carbohydrates (35%). The third feed consisted of fish meal and fish oil, was rich in soluble proteins (47%) and was referred to as "animal feed." The level of soluble proteins, carbohydrates, and lipids in the feeds was determined using the techniques of Lowry et al. (1951), Dubois et al. (1956), and Folch et al. (1957), respectively. Ash was measured by placing the dried tissues in a muffle furnace at 500°C for 4 h. The energetic values were calculated by multiplying the level of each organic constituent by its energy equivalent (Brody 1945). Feeds were prepared by progressively adding the meal, oil, and vitamin mix-

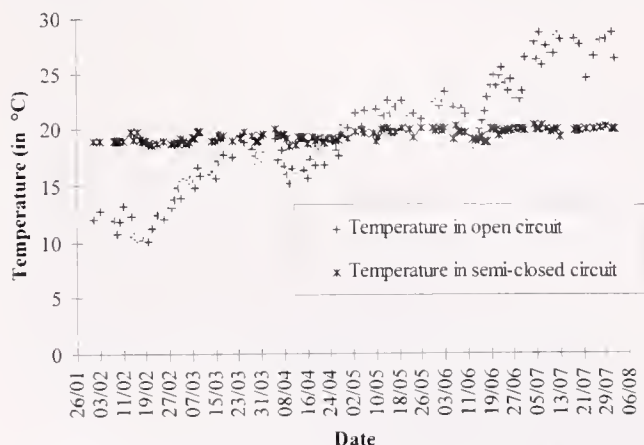


Figure 1. Evolution of water temperature within the tanks (open and semiclosed circuits) throughout the 6-month rearing period.

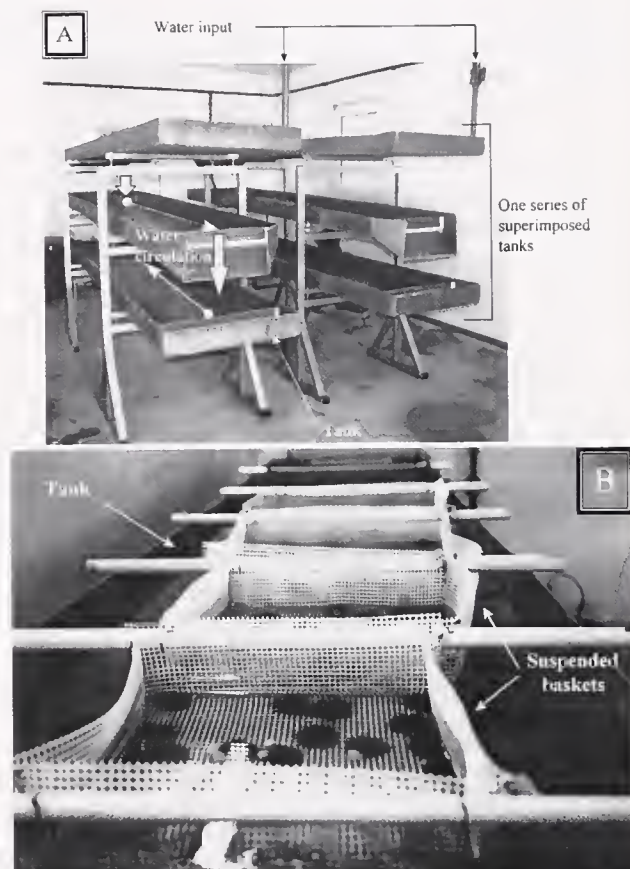


Figure 2. A. Photograph of two series of superimposed tanks (open circuit), with water circulation. B. Photograph of plastic suspended baskets in which sea urchins are placed (30 individuals per basket).

ture to the binder (gelatin previously dissolved in water, 12.5% solution). The paste thus obtained was spread onto a plaque at a thickness of 1.5 cm. Once hardened, this food plaque was cut into cubes of 1 cm × 1 cm × 1.5 cm. Sea urchins were always provided surplus food.

Each tank was assigned particular environmental conditions and a specific diet. The exact characteristics of the different tanks are provided in Table 2. For increased clarity, the tanks were given a three letter code. Each letter represents one of the studied treatments. The first letter corresponded to the nature of the food distributed: V = vegetable feed; M = mixed formulated feed; A = animal formulated feed; C = *Cymodocea nodosa* fronds. The second letter indicated the rearing temperature: R = temperature maintained at 18 to 20°C (in the semiclosed circuit); and N = natural temperature (in the open circuit). The third letter corresponded to the light conditions: L = natural light; and D = total darkness.

Growth Parameters

Rearing began on February 1, 1994. The experiment lasted 6 months. Each month the test diameter (without the spines) of all the individuals was measured using a sliding caliper. At the end of the rearing period (August 1994), 10 sea urchins of the 40 to 45, 30 to 35, and 20 to 25 mm size classes were sampled from each of the tanks. These sea urchins were measured and weighed. They were then dissected into various components: gonads, gut (without

TABLE 1.

Ingredients used in the preparation of the formulated feeds (expressed as a percentage).

	Vegetable Feed	Animal Feed	Mixed Feed
Corn meal	44.7%	0.0%	22.35%
Wheat Meal	44.7%	0.0%	22.35%
Fish meal ^a	0.0%	89.4%	44.70%
Sunflower oil	8.9%	0.0%	4.45%
Cod liver oil	0.0%	8.9%	4.45%
Vitamin and mineral mixture ^b	1.7%	1.7%	1.70%
Soluble proteins	12.7%	28.9%	47.2%
Soluble carbohydrates	58.2%	35.3%	15.9%
Total lipids	10.7%	12.8%	15.5%
Ash	4.9%	8.1%	10.7%
Energy level	17.1	18.0	19.8

The formulated feed contain 56% of base meal mixed into a 12.5% gelatin solution. Biochemical composition is also expressed as a percentage of dry weight. Energetic levels are expressed in kJ.g⁻¹ dry weight.

^a The meal was produced by Norsildmel[®] and is made up of fresh cold water Norwegian fish (herring, capelin, mackerel).

^b The mixture is made up of (expressed in mg or UI/kg of feed): tocopherol acetate: 70.8 UI, ascorbic acid: 283 mg; thiamin: 7.1 mg; riboflavin: 7.6 mg; pyridoxine: 9.4 mg; cyanobalanine: 0.014 mg; biotine: 0.47 mg; folic acid: 1.89 mg; calcium pantothenate: 23.6 mg; vit. A: 710 UI; vit D₃: 700 UI; niacin: 14.6 mg; CaCO₃: 2.1 mg; Cu SO₄: 9.4; Fe SO₄: 4.7 mg; NaF: 7.1 mg; Mg CO₃: 174 mg; Mn SO₄: 18.9 mg; CaHPO₄: 75.5 mg; Zn SO₄: 7.7 mg.

gut contents), test (without Aristotle's lantern), Aristotle's lantern, and gut contents. Each component was drained on filter paper and weighed to the nearest 0.1 mg. The relation between components was then calculated. CI (component index) is the ratio between the wet weight of the component (WWC) expressed in mg and the total wet weight of the sea urchin (TWW) expressed in mg: $CI (\%) = WWC \times 100/TWW$ (Lawrence et al. 1965).

Statistics

The growth for each treatment and size class was analyzed using a simple linear regression (Zar 1984). The goodness of fit was tested by the Chi square test or linearity test. Differences in the rate of growth were demonstrated using an analysis of covariance followed by a multiple comparisons among slopes (Zar 1984). The comparisons of mean sizes and physiological indices as a function of treatment were processed by one-way or two-way analysis of variance (ANOVA) followed by Tukey tests (Zar 1984). Previously, normality and homoscedasticity were verified by Kolmogorov-Smirnov and Bartlett tests, respectively (Zar 1984). The software Statgraphics Plus (v. 2.1) for Windows was used. For those treatments where the five size classes were present, the growth equations given by von Bertalanffy (1938) were used. The model parameters were estimated using the method of Gulland and Holt (1959) as modified by Lockwood (1974). This linear method was chosen, because it allows growth measurements to be obtained without having to determine the age of the individuals.

RESULTS

Rearing mortality was fairly low (7.5%) throughout the 6 months of the experiment. These mortalities can be attributed to a

temporary (1 day) decrease in the flow of incoming water in one tank (CNL) which brought about an oxygen shortage.

Growth Rate

The monthly growth rates in terms of test diameter for the eight diets and rearing treatments and five size classes are given in Table 3. For the calculation of mean monthly growth, the data for each of the paired basket were pooled (60 sea urchins/treatment). Indeed, for each treatment and size class, the paired sets exhibited an identical mean size and weight at each of the data acquisition periods (Student's *t*-tests, $t = 0.4$ to 1.6 , $df = 46$ to 58 , $p > .05$). The results revealed that growth varied as a function of the treatment, time of year and sea urchin size [ANOVA and analysis of covariance (ANCOVA) $p < .05$].

The evolution in mean size in the eight tanks exhibited a linear tendency (Table 4) (growth curves are usually logarithmic, but over relatively short periods of time (6 months) the curve takes on a linear appearance). The statistical analyses revealed that growth was significantly linear, and this was regardless of the treatment and size class examined (testing for linearity, $F = 0.08$ to 3.80 , $p > .05$). In addition, the slopes were all positive (testing for slope, $F = 19.1$ to 737 , $p < .05$). The rate of growth for each size class, as represented by the slopes of the linear regressions, varied according to treatment (ANCOVA, $F = 9.8$ to 35.1 , $p < .05$). The results of the multiple comparison among slopes test performed *a posteriori* are presented in Table 4. Generally, the slowest growth was obtained in tanks CNL, VRL, and VNL (vegetable feed); whereas, the fastest growth occurred in tanks MNL and ANL (animal or mixed feed).

Two environmental parameters were examined; namely, temperature and light. Regardless of size class and diet, growth rates were lower in the semiclosed circuit (18–20°C) than in the open circuit at ambient environmental temperatures (slope comparison test, $p < .05$). At ambient environmental temperatures, the lowest growth was usually observed in winter (February); whereas, the highest growth occurred in June (ANOVA, $F = 11.2$ to 45.6 , $p < .05$). The general tendency seems to be toward an increase in growth when temperature is in the vicinity of 20°C. To demonstrate this result, a correlation was made between growth rate and the difference between tank temperatures and the optimal growth temperature in *Paracentrotus lividus* (20°C). The results obtained for each treatment reveal that only half of the relationships established are significant ($r > 0.90$). Statistical analysis of the data concerning the effects of light revealed that total darkness did not result in any significant differences in growth as compared to light conditions (slope comparison test, $p > .05$ for all size classes).

The results also indicated that a significant negative correlation existed between total growth and the initial mean size of the individuals (all treatments considered) ($y = -0.156x + 8.132$, $r = 0.90$, $n = 30$; testing of correlation coefficient, $p < .05$). The ANCOVA performed, for each treatment, among the different size classes indicated that the 40 to 45 mm size class exhibited the lowest growth regardless of treatment. The next two size classes, in increasing order of growth, were 30 to 35 mm and 20 to 25 mm. The rate of growth for the 3 to 10 mm and 10 to 15 mm size classes were identical and were the highest recorded of the five size classes considered.

Growth Modeling

The modeling of *Paracentrotus lividus* growth under rearing conditions was performed using the data generated by providing

TABLE 2.
Rearing conditions in the different land-based tanks.

Tank VRL	Tank VNL	Tank CNL
Vegetable formulated diet (V)	Vegetable formulated diet (V)	<i>Cymodocea nodosa</i> diet (C)
Regulated temperature (18–20°) (R)	Natural temperature (N)	Natural temperature (N)
Natural light (L)	Natural light (L)	Natural light (L)
Size class 20 to 25 mm	Size class 20 to 25 mm	Size class 20–25 mm
Size class 30 to 35 mm	Size class 30 to 35 mm	Size class 30–35 mm
Size class 40 to 45 mm	Size class 40 to 45 mm	Size class 40–45 mm
Tank MRL	Tank MNL	Tank ANL
Mixed formulated diet (M)	Mixed formulated diet (M)	Animal formulated diet (A)
Regulated temperature (18–20°) (R)	Natural temperature (N)	Natural temperature (N)
Natural light (L)	Natural light (L)	Natural light (L)
Size class 3 to 9 mm	Size class 3 to 9 mm	Size class 20 to 25 mm
Size class 10 to 15 mm	Size class 10 to 15 mm	Size class 30 to 35 mm
Size class 20 to 25 mm	Size class 20 to 25 mm	Size class 40 to 45 mm
Size class 30 to 35 mm	Size class 30 to 35 mm	
Size class 40 to 45 mm	Size class 40 to 45 mm	
Tank MRD	Tank MND	
Mixed formulated diet (M)	Mixed formulated diet (M)	
Regulated temperature (18–20°) (R)	Natural temperature (N)	
Darkness (D)	Darkness (D)	
Size class 3 to 9 mm	Size class 3 to 9 mm	
Size class 10 to 15 mm	Size class 10 to 15 mm	
Size class 20 to 25 mm	Size class 20 to 25 mm	
Size class 30 to 35 mm	Size class 30 to 35 mm	
Size class 40 to 45 mm	Size class 40 to 45 mm	

the mixed formulated feed (by using the five size classes, we can represent the different size classes present in the lagoon). Two models were drawn up: the first makes use of the data obtained at the regulated temperature (semiclosed circuit, data from tanks MRD and MRL were pooled). The second model was drawn up using the data obtained at environmental temperatures (open circuit, data from tanks MND and MNL). The method described by Gulland and Holt (1959), applied to the five size classes for each of the temperature treatments, allowed the parameters of two von Bertalanffy equations to be calculated (Fig. 3); (1) for those individuals reared at the regulated temperature : $k = 0.0245$; $L_{\infty} = 52.355$; $t_0 = -2.409$; (2) for sea urchins maintained at environmental temperatures : $k = 0.0252$; $L_{\infty} = 59.480$; $t_0 = -2.053$.

The validity of the models, drawn up using the data generated by the rearing experiment, was tested by performing a linear regression between the experimental and theoretic data (these last being calculated from the models' equations). The correlation and linearity were significant ($r = 0.99$ and linearity test, $p > .05$; for both models). In addition, the slopes and linear regressions obtained were both not significantly different from 1 (comparison test between the slope obtained and a theoretic slope of 1, $p > .05$ in both cases). It can, therefore, be concluded that the two theoretic models are in accordance with the data generated experimentally.

Physiological Indices

The relative importance of the various components of the individuals taken from the eight tanks is represented in Figure 4. Statistical analysis of the gonadal index reveals that important

variations in this index occurred as a function of both "diet-rearing" treatment and size class. In addition, a significant interaction existed between these two factors (two-way ANOVA, "diet-rearing" factor : $F = 33.00$, $p < .001$; size factor : $F = 18.16$, $p < .001$; interaction, $F = 4.68$, $p < .001$). This significant interaction signifies that the evolution in gonadal index as a function of size differs depending upon the "diet-rearing" regime. The gonadal indices for the 20 to 25 mm size class were very high and were even above those of the larger individuals (in particular in tanks MNL and MND; one-way ANOVA, $F = 4.1$ and 7.7 , respectively; and Tukey tests, $p < .05$). Conversely, in the CNL tank (where individuals were fed *Cymodocea nodosa*), the gonadal index/sea urchin size ratio was identical to that observed in sea urchins living in the lagoon. The differences observed among the different rearing tanks allows three distinct groups to be distinguished. The first was composed of individuals fed *Cymodocea nodosa*, the second was individuals reared in tanks VRL, MRL, and MRD (regulated temperature), and the third group (having the highest gonadal index) contained those individuals reared in the tanks VNL, MND, ANL, and MNL (environmental temperatures); (one-way ANOVA, $F = 8.8$ to 21.6 ; and Tukey tests, $p < .05$). The relative importance of the gut, test, and lantern in the reared sea urchins did not differ significantly as a function of the "diet-rearing" treatment administered (two-way ANOVA, "diet-rearing" factor : $F = 1.38$, 1.34 , and 1.47 respectively, $p > .05$ in all three cases). Conversely, a significant difference in these three components was observed as a function of size class (size class factor : $F = 29.99$, 43.01 , and 55.67 respectively, $p < .001$). The interaction between these two

TABLE 3.

Mean monthly growth rate (\pm standard error) of reared *Paracentrotus lividus* (in mm/month) for the eight treatments and five size classes.

		Feb 94	March 94	April 94	May 94	June 94	July 94	Total
40 to 45 mm	VRL	0.09 \pm 0.26	0.05 \pm 0.27	0.27 \pm 0.26	0.35 \pm 0.27	0.18 \pm 0.27	0.14 \pm 0.26	1.07 \pm 0.26
	MRL	0.14 \pm 0.23	0.24 \pm 0.24	0.31 \pm 0.26	0.15 \pm 0.25	0.43 \pm 0.26	0.31 \pm 0.28	1.58 \pm 0.26
	MRD	0.10 \pm 0.28	0.20 \pm 0.30	0.36 \pm 0.30	0.18 \pm 0.29	0.47 \pm 0.28	0.32 \pm 0.28	1.63 \pm 0.27
	VNL	0.11 \pm 0.27	0.25 \pm 0.27	0.25 \pm 0.27	0.22 \pm 0.29	0.37 \pm 0.30	0.09 \pm 0.30	1.29 \pm 0.28
	MNL	0.24 \pm 0.30	0.39 \pm 0.33	0.50 \pm 0.36	0.17 \pm 0.38	0.75 \pm 0.38	0.26 \pm 0.38	2.31 \pm 0.33
	MND	0.14 \pm 0.28	0.29 \pm 0.28	0.38 \pm 0.28	0.21 \pm 0.30	0.40 \pm 0.31	0.40 \pm 0.31	1.82 \pm 0.29
30 to 35 mm	CNL	0.12 \pm 0.28	0.28 \pm 0.31	0.32 \pm 0.33	0.10 \pm 0.35	0.32 \pm 0.35	0.16 \pm 0.32	1.30 \pm 0.29
	ANL	0.18 \pm 0.27	0.64 \pm 0.26	0.77 \pm 0.26	0.05 \pm 0.27	0.53 \pm 0.28	0.19 \pm 0.27	2.36 \pm 0.27
	VRL	0.21 \pm 0.29	0.30 \pm 0.29	0.06 \pm 0.30	0.29 \pm 0.31	0.76 \pm 0.33	0.12 \pm 0.33	1.74 \pm 0.32
	MRL	0.30 \pm 0.29	0.46 \pm 0.31	0.49 \pm 0.33	0.27 \pm 0.33	0.85 \pm 0.33	0.72 \pm 0.34	3.09 \pm 0.32
	MRD	0.17 \pm 0.27	0.58 \pm 0.29	0.60 \pm 0.31	0.18 \pm 0.32	0.86 \pm 0.33	0.75 \pm 0.33	3.14 \pm 0.29
	VNL	0.25 \pm 0.29	0.46 \pm 0.30	0.69 \pm 0.30	0.39 \pm 0.31	0.80 \pm 0.34	0.41 \pm 0.37	3.00 \pm 0.33
20 to 25 mm	MNL	0.52 \pm 0.29	0.62 \pm 0.30	0.91 \pm 0.31	0.46 \pm 0.31	1.74 \pm 0.33	0.36 \pm 0.35	4.61 \pm 0.32
	MND	0.52 \pm 0.29	0.71 \pm 0.29	0.87 \pm 0.31	0.50 \pm 0.31	0.69 \pm 0.31	0.21 \pm 0.31	3.50 \pm 0.30
	CNL	0.29 \pm 0.28	0.61 \pm 0.29	0.37 \pm 0.33	0.37 \pm 0.35	0.67 \pm 0.36	0.36 \pm 0.36	2.67 \pm 0.45
	ANL	0.63 \pm 0.27	1.01 \pm 0.29	0.87 \pm 0.30	0.55 \pm 0.32	0.84 \pm 0.34	0.64 \pm 0.36	4.54 \pm 0.32
	VRL	0.71 \pm 0.30	0.35 \pm 0.31	0.07 \pm 0.30	0.42 \pm 0.28	0.22 \pm 0.33	0.39 \pm 0.30	2.17 \pm 0.29
	MRL	0.22 \pm 0.27	0.74 \pm 0.27	0.62 \pm 0.28	0.21 \pm 0.31	0.69 \pm 0.34	0.74 \pm 0.34	3.22 \pm 0.31
10 to 15 mm	MRD	0.52 \pm 0.30	0.64 \pm 0.31	0.95 \pm 0.31	0.37 \pm 0.31	0.83 \pm 0.30	0.81 \pm 0.28	4.12 \pm 0.29
	VNL	0.37 \pm 0.31	0.52 \pm 0.32	0.61 \pm 0.34	0.50 \pm 0.35	0.80 \pm 0.34	0.45 \pm 0.32	3.25 \pm 0.31
	MNL	0.42 \pm 0.29	1.12 \pm 0.30	1.26 \pm 0.30	0.62 \pm 0.31	1.27 \pm 0.32	0.45 \pm 0.33	5.14 \pm 0.32
	MND	0.39 \pm 0.30	0.88 \pm 0.29	1.48 \pm 0.30	0.47 \pm 0.31	1.49 \pm 0.31	0.12 \pm 0.34	4.83 \pm 0.34
	CNL	0.30 \pm 0.28	0.88 \pm 0.26	0.81 \pm 0.31	0.54 \pm 0.35	0.60 \pm 0.37	0.15 \pm 0.37	3.28 \pm 0.34
	ANL	0.66 \pm 0.26	1.16 \pm 0.25	1.24 \pm 0.24	0.96 \pm 0.25	0.90 \pm 0.26	0.87 \pm 0.25	5.79 \pm 0.25
3 to 9 mm	MRL	0.53 \pm 0.23	0.60 \pm 0.21	1.43 \pm 0.27	0.62 \pm 0.32	1.38 \pm 0.34	1.12 \pm 0.34	5.68 \pm 0.29
	MRD	0.49 \pm 0.25	0.61 \pm 0.24	1.26 \pm 0.28	0.60 \pm 0.31	1.39 \pm 0.31	1.21 \pm 0.37	5.56 \pm 0.34
	MNL	0.64 \pm 0.25	1.53 \pm 0.24	1.72 \pm 0.26	1.16 \pm 0.31	1.84 \pm 0.36	0.57 \pm 0.38	7.46 \pm 0.33
	MND	0.54 \pm 0.25	1.47 \pm 0.23	1.47 \pm 0.28	1.38 \pm 0.35	1.53 \pm 0.39	0.66 \pm 0.42	7.05 \pm 0.36
	MRL	0.82 \pm 0.45	0.66 \pm 0.47	1.85 \pm 0.52	1.35 \pm 0.58	0.70 \pm 0.58	1.01 \pm 0.61	6.39 \pm 0.54
	MRD	1.05 \pm 0.42	0.39 \pm 0.41	1.25 \pm 0.45	1.36 \pm 0.48	1.32 \pm 0.47	1.26 \pm 0.59	6.63 \pm 0.56
	MNL	0.57 \pm 0.45	1.09 \pm 0.47	1.79 \pm 0.43	1.47 \pm 0.41	1.09 \pm 0.50	0.93 \pm 0.62	6.94 \pm 0.54
	MND	0.45 \pm 0.34	0.93 \pm 0.39	2.32 \pm 0.50	1.64 \pm 0.69	1.17 \pm 0.70	0.39 \pm 0.58	6.9 \pm 0.46

Tank codes, the letter corresponds to the food: V: vegetable feed; M: mixed feed; A: animal feed; C: *Cymodocea nodosa*. The second letter indicates the rearing temperature; R: temperature maintained at 18 to 20°C; N: natural temperature. The third letter corresponds to the light conditions: L: natural light; D: total darkness.

factors ("diet-rearing" and size class) was not significant, indicating that the variations observed among the different size classes were identical for each of the tanks. Individuals of the 40 to 45 mm size class possessed a gut index that was lower than that observed for the two other size classes. The lowest test index was observed for the 40 to 45 mm size class, followed by the 30 to 35 size class and, finally, the highest value was seen for the 20 to 25 mm size class (Tukey test, $p < .05$). The lowest lantern index was observed for the 40 to 45 size class followed by the 30 to 35 mm size class, and, finally, the 20 to 25 mm size class (Tukey tests, $p < .05$).

DISCUSSION

Effect of Food Type on Growth

Statistical analysis of all of the size classes revealed that food type had a significant effect on the rate of growth. The highest growth rate was obtained using the mixed and animal feeds. The growth observed was higher than has been found by a number of authors (Allain 1978, Le Gall and Bucaille 1987, Azzolina 1988, Turon et al. 1995), particularly for the 30 to 35 mm size class. A number of studies have demonstrated that growth rates in the Echinoidea differ with the food type available and/or ingested (quality

and quantity) (Lawrence 1975, Vadas 1977, Rowley 1990, Frantzis and Grémare 1992). Although *Cymodocea nodosa* is considered to be the preferred food source of *Paracentrotus lividus* (Traer 1980), the use of this food type in the present study did not yield maximal growth. It should be noted, however, that the decrease in oxygen levels (for 1 day) in the CNL tank may have led to a temporary decrease in growth rate and, thus, lower the total growth observed during the 6-month rearing period. The increase in sea urchin growth rates when fed formulated feeds (mixed or animal) as compared to the growth observed on a diet made up of *Cymodocea nodosa* has been observed in a previous study using similar rearing conditions (Fernandez and Caltagirone 1994, Fernandez 1996). The present study, therefore, confirms that the quality of the mixed and animal formulated feeds allows growth to be maximized. This food quality is fairly difficult to assess, because it encompasses a number of characteristics, such as quantity, digestibility, absorption, and composition of the food (Lawrence and Lane 1982). A possible first observation concerns somatic growth, which was always higher for these two feeds, the biochemical composition of which was characterized by a high level of soluble proteins. In fact, it has been demonstrated that the growth of certain Echinoidea depends upon the levels of ingested protein (Lowe and

TABLE 4.

Comparison of equation parameters describing the increase in mean test size (y, in mm) as a function of rearing time (x, in days) for sea urchins based on their initial size and rearing treatment.

	3 to 9 mm	10 to 15 mm	20 to 25 mm	30 to 35 mm	40 to 45 mm
Tank VRL	—	—	b = 0.0109 ^a a = 23.687 r = 0.96	b = 0.0101 ^a a = 32.428 r = 0.99	b = 0.0066 ^a a = 41.437 r = 0.86
Tank MRL	b = 0.0377 ^a a = 5.9296 r = 0.99	b = 0.0325 ^a a = 13.9896 r = 0.99	b = 0.0179 ^b a = 23.511 r = 0.98	b = 0.017 ^b a = 32.389 r = 0.99	b = 0.0089 ^{ab} a = 41.511 r = -0.98
Tank MRD	b = 0.0366 ^a a = 5.6176 r = 0.99	b = 0.0314 ^a a = 13.932 r = 0.99	b = 0.0231 ^c a = 23.404 r = 0.99	b = 0.0175 ^b a = 32.368 r = 0.98	b = 0.0094 ^{ab} a = 41.366 r = 0.95
Tank VNL	—	—	b = 0.0189 ^{bc} a = 23.304 r = 0.97	b = 0.0178 ^{ab} a = 32.351 r = 0.99	b = 0.0078 ^a a = 41.367 r = 0.97
Tank MNL	b = 0.0419 ^b a = 5.8804 r = 0.99	b = 0.0451 ^b a = 13.8 r = 0.99	b = 0.0309 ^d a = 23.25 r = 0.99	b = 0.0271 ^{cd} a = 32.286 r = 0.98	b = 0.0134 ^c a = 41.394 r = 0.98
Tank MND	b = 0.0440 ^b a = 5.673 r = 0.98	b = 0.0427 ^b a = 13.804 r = 0.99	b = 0.0299 ^{cd} a = 23.184 r = 0.98	b = 0.0208 ^{bc} a = 32.593 r = 0.99	b = 0.0103 ^b a = 41.497 r = 0.98
Tank CNL	—	—	b = 0.0201 ^{bc} a = 23.376 r = 0.97	b = 0.0153 ^{ab} a = 32.538 r = 0.94	b = 0.0064 ^a a = 41.695 r = 0.80
Tank ANL	—	—	b = 0.0306 ^d a = 23.051 r = 0.99	b = 0.0259 ^d a = 32.612 r = 0.99	b = 0.0143 ^c a = 41.466 r = 0.98

For all size classes $n = 14$, except for the 3 to 9 mm class size, where $n = 7$; b: the slope [the results of the multiple comparison among slopes (for each size class) is given by an exponent letter: values that do not differ at the 0.05 level are noted with a same letter]; a: intercept, r: correlation coefficient. Tank codes, the letter corresponds to the food: V: vegetable feed; M: mixed feed; A: animal feed; C: *Cymodocea nodosa*. The second letter indicates the rearing temperature: R: temperature maintained at 18 to 20°C, N: natural temperature. The third letter corresponds to the light conditions: L: natural light, D: total darkness.

Lawrence 1976, Frantzis and Grémare 1992). The results presented here would, therefore, seem to be in agreement with these studies. They also reveal that formulated feeds made up of fish meal represent good food sources for *Paracentrotus lividus*. It should also be noted that an increase in the percentage of fish meal in the feed (which causes an increase in the level of protein) does not, in the present study, bring with it a significant increase in somatic growth. Part of the energy used must, therefore, be lost or used to other ends (reproduction, building of reserves).

Effect of Type Water System (Open Circuit/Semiclosed Circuit)

An attempt was made to compare growth rates obtained in the open circuit (natural environmental temperatures) with those obtained in the semiclosed circuit (regulated temperature). It is obvious that such a comparison between two very different water systems is only of value as a simple observation. Nevertheless, the results obtained are interesting. Indeed, these results (lower growth rates at the regulated than at nonregulated temperatures) are in contradiction with pre-existing data on *Paracentrotus lividus*. Indeed, Le Gall et al. (1990) suggested that the growth of *Paracentrotus lividus* was at a maximum at 18 to 20°C. In addition, it has been demonstrated that maximal growth of *Paracentrotus lividus* in Urbino lagoon occurs when water temperature is also approximately 18 to 20°C (Fernandez 1996). It can, therefore, be hypothesized that factors other than temperature affected sea urchin growth. Under rearing conditions, the pollution generated by the

rearing itself (disintegration of food, biodegradation of this disintegrated food, and urchin metabolic waste) is a parameter that would seem to be of some importance. In the present study, the water renewal in the semiclosed circuit was voluntarily low in order to lower the energy costs associated with regulating the rearing temperature, and a decanting tank was the only means used to eliminate a portion of the suspended matter. As a result, nitrite peaks were sometimes observed (>0.2 mg/L), the level of which were above the level generally considered detrimental to marine invertebrates (<0.1 mg/L, Alzieu 1989). The accumulation of rearing waste products (ammonia and nitrite) are toxic to the animals and increases their oxygen demand (Gonzalez et al. 1993) and, thus, represent stress factors capable of inhibiting growth. Grosjean et al. (1996) have observed that, under rearing conditions, a number of individuals exhibit very low growth rates (usually generating a multimodal size frequency distribution) and concluded that the lower productivity of the small individuals is not genetically induced but rather the result of poor water quality. The low growth observed in the semiclosed circuit may, therefore, be partially attributable to the poor water quality present in these tanks.

Effect of Variations in Water Temperature on Growth in the Open Circuit of Temperature

The growth of sea urchins in the open circuit differs substantially from months (Table 3). Water temperature in the open

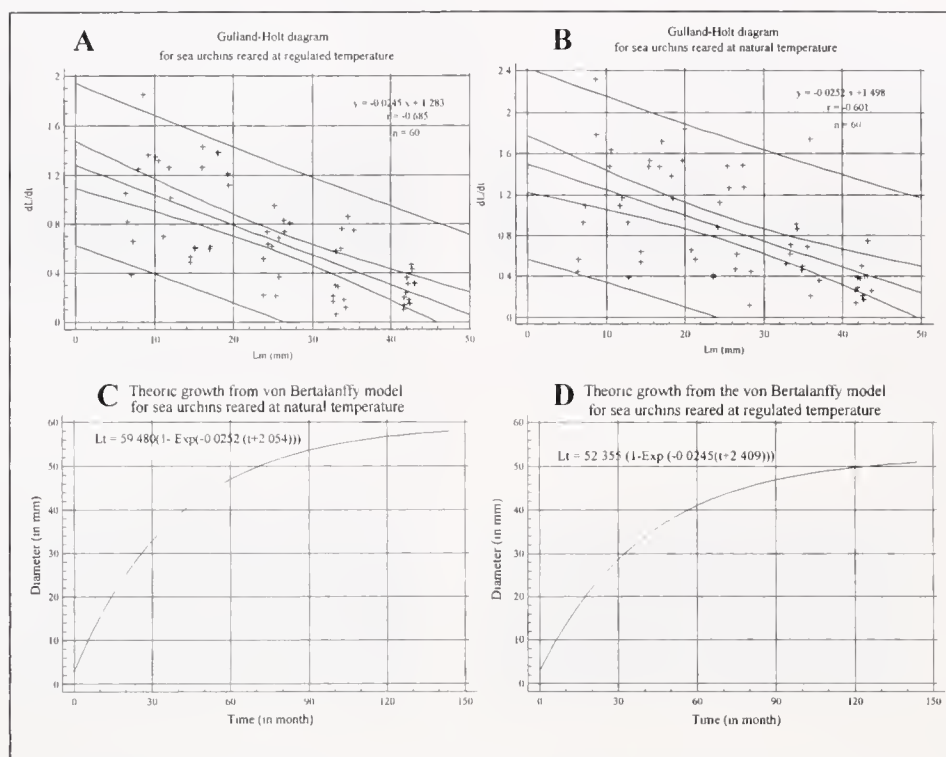


Figure 3. Calculation of the growth models: A and B. Gulland-Holt diagrams used to determine the parameters for the von Bertalanffy model generated based on the growth data obtained for sea urchins reared at both natural and regulated temperatures. C and D. The von Bertalanffy equations produced.

circuit presents the same seasonal variations as those observed *in situ* in the lagoon (from 9–29°C, Fernandez 1996). A number of authors have noted the importance of temperature on the growth of Echinoidea (Le Gall et al. 1990, Lares and McClintock 1991, Guillou and Michel 1994). The rearing of *Paracentrotus lividus* in the natural environment (immersed cages *in situ*) has demonstrated that growth is dependent on temperature (Fernandez 1996) with a general tendency that seems to be toward an increase in growth when temperature is in the vicinity of 20°C. The high proportion of nonsignificant relationships observed (between growth and temperature) may be attributable to the low number of datapoints available ($n = 5$). In addition, it should be noted that these correlations have been drawn after eliminating the growth value for the month of May (for all tanks and size classes). These growth values are very low when the temperature is 19.1°C. It should be noted that the month of May was characterized by several massive and successive spawning events (May 3, 6, and 17). All of the size classes were involved in these spawns and this in all of the tanks (whether in open or semiclosed circuits). It is possible that after these spawning events, a portion of the energy ingested went toward gonadal production to replenish the gonad and gametes, and this at the expense of somatic growth. These results reveal that temperature was not the sole parameter responsible for the observed seasonal variations in growth. The reproductive cycle seems to be one of the more important parameters. Indeed, the low somatic growth observed during the spawning period would seem to be in agreement with the observations made by a number of researchers. The latter have observed an inverse relationship between gonadal and somatic growths (Vadas 1977, Greenwood 1980, Lawrence and Lane 1982, Azzolina 1988, Guillou and

Michel 1994). In the present study, it was not possible to correlate gonadal and somatic growths, because the cycle of the gonadal index was not recorded in the tanks. Indeed, the substantial differences existing between the *in situ* and reared gonadal indices was such as to make an extrapolation of the latter values impossible to perform. The last parameter to have an effect on somatic growth is the sea urchin's ingestion levels. Indeed, this parameter varies with sampling month, and these variations are correlated with those observed for growth (Fernandez 1996, Fernandez and Boudouresque 1997). Guillou and Michel (1994) also observed that the resumption of growth in *Sphaerechinus granularis* (Lamarck, 1816) is attributable, at least in part, to a resumption in feeding activity (because the repletion index is correlated to temperature). In conclusion, the variations in somatic growth observed in the tanks would seem to be linked to a number of interacting factors including temperature, reproductive cycle, and ingestion.

Effect of Light on Growth

Several species of sea urchins, including *Paracentrotus lividus*, are very active at night (Gamble 1966, Shepherd and Boudouresque 1979, Dance 1987) especially in so far as trophic activity is concerned (Powis de Tembossche 1978, Rico et al. 1990). Grosjean and Jangoux (1994) have observed a higher level of ingestion and absorption in individuals of this species reared in total darkness (this implies a higher level of food available for growth). In light of this, Le Gall (1989) has proposed rearing sea urchins in total darkness in order to increase somatic growth. Pearse et al. (1995) and Beyer et al. (1998) have also observed

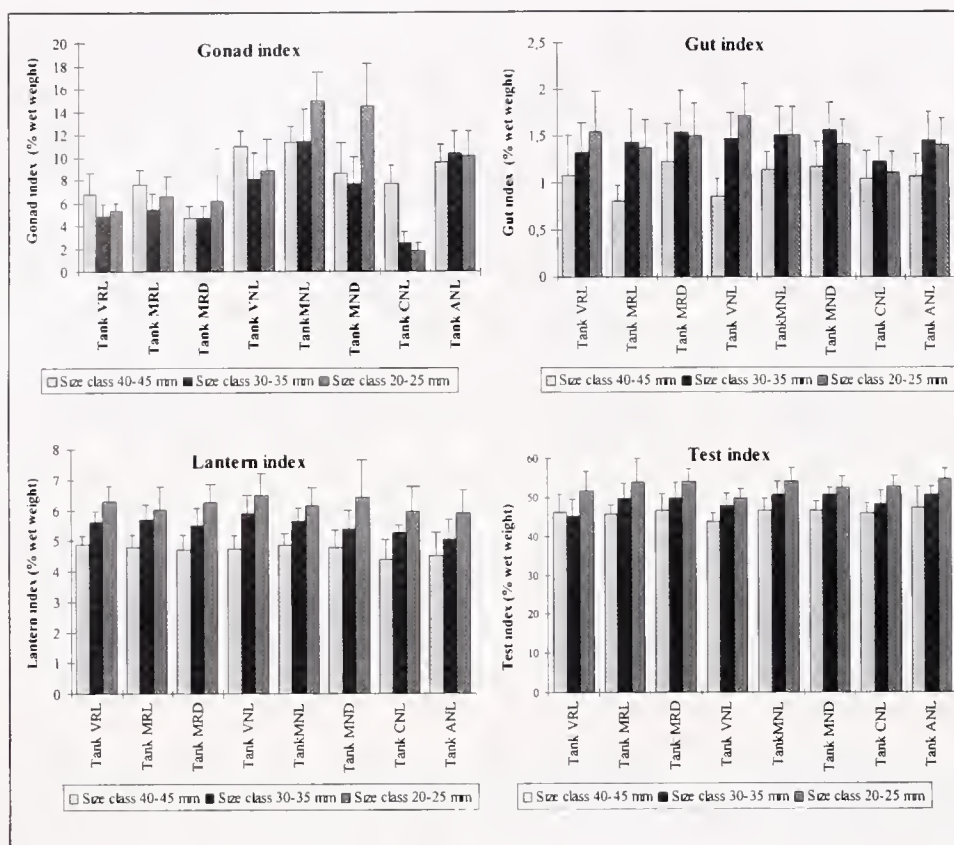


Figure 4. Mean gonadal, gut, test, and lantern indices ($\pm 95\%$ confidence interval) for three size classes of *Paracentrotus lividus* reared in tanks and fed either formulated feeds or *Cymodocea nodosa*. Tank codes, the first letter corresponds to the food: V: vegetable feed; M: mixed feed; A: animal feed; C: *Cymodocea nodosa*. The second letter indicates the rearing temperature: R: temperature maintained at 18 to 20°C; N: natural temperature. The third letter corresponds to the light conditions: L: natural light; D: total darkness.

greater somatic growth and a low gonadal growth in *Strongylocentrotus fransiscanus* (Stimpson 1857) and *Strongylocentrotus purpuratus* (Stimpson 1857) when placed under dark conditions as compared to individuals reared in continuous light. The results obtained here do not confirm these results: total darkness did not bring about an increase in somatic growth as compared to continuous light conditions. Dark conditions in the present study were obtained by placing an opaque black cover over the appropriate tanks (tanks MRD and MLD). It was necessary to remove the cover twice a week for a period of about 20 minutes in order to clean the tanks. This operation was always performed at night using a low watt formulated light. Nevertheless, it is possible that these cleaning operations disrupted the experiment. Indeed, Grosjean and Jangoux (1994) observed that the sea urchin food ration was lowest when the individuals were subjected to a 12 h light/12 h dark photoperiod, and they attributed this phenomenon to the sudden passage from light to dark conditions (Grosjean pers. comm.). It is possible that in the present study the sudden passage from dark to light conditions was disrupting and, thus, did not allow an increase in growth to be observed.

Effect of Initial Size on Growth

Growth in the Echinoidea, such as *Paracentrotus lividus*, varies as a function of age and, therefore, size of the individuals (cf. growth models: Allain 1978, Fenaux et al. 1987, Azzolina 1988, Turon et al. 1995). In the present study, five different size classes

were used. The results generated revealed that, for the 40 to 45 mm size class, growth is very low and is most probably not profitable in a commercial aquaculture context. Indeed, despite the fact that large sea urchins ingest important quantities of food, this does not allow for a rapid or significant size increase. Although small sea urchins are not commercialized, it would seem that the rationalization of sea urchin aquaculture necessitates that rearing be stopped once the urchins have reached a size of 40 mm (this being the minimum size required for the commercialization of this species).

Growth Curves

The models presented here must be considered with a certain degree of caution. Because no individuals larger than 45 mm were examined, the model is not valid beyond this size. In addition, growth was studied over a period of only 6 months (February–July 1994). Therefore, the seasonal variations in growth have not been included in the models. Nevertheless, the models presented are quite similar to those reported by other authors (Fig. 3) and, in particular, when the von Bertalanffy model was used (Allain 1978, Azzolina 1988). The growth curves calculated for natural temperature present a growth more rapid than those generated by other models (with the exception of the logistic model proposed by Fenaux et al. 1987), at least for the size range considered in the present study (3–45 mm).

Physiological Indices

From an aquacultural point of view, what is important is the gonadal index. Indeed, the gonadal index usually increases with increasing sea urchin size (Fuji 1967, Nichols et al. 1985, Pearse and Cameron 1991, Lumingas 1994) and this even in lagoon environments (Fernandez and Boudouresque 1997). The results obtained in the tanks of the present study were in direct contradiction with what is reported in the literature: namely, the gonadal indices for the 20 to 25 mm size classes were very high and even superior to those observed in larger individuals (particularly in tanks MNL and MND). Conversely, in the CNL tank (individuals fed *Cymodocea nodosa*), the gonadal index/sea urchin size ratio was identical to that observed in wild individuals. The rearing of sea urchins in tanks using a formulated diet, therefore, causes substantial changes in gonadal growth. The energy allocated to gonad development was, therefore, more important under rearing conditions, especially in small sized individuals (20–25 mm), than was observed in the natural environment. In *Evechinus chloroticus* (Valenciennes), Barker (pers. comm.) also observed high gonadal indices in small individuals. The increase in gonad weight may, in part, be attributable to an increase in nutritive phagocytes rather than gametes (Walker 1982, Pearse and Cameron 1991). As a general rule, once a portion of the energy has been allocated to maintenance, the energy remaining is divided between reproduction and somatic growth (Calow 1981). In the small individuals of the present study (in particular in the MNL and MND tanks), a large amount of energy, therefore, remains available for reproduction that leads to gonads the weight of which were very high for these size classes.

In so far as the treatment differences are concerned, the results revealed that over a long time period (6 months), all of the food types brought about high gonadal growth. Finally, it should be noted that the gonadal index generated under rearing conditions was higher than that observed in wild sea urchins at the same time of year (Fernandez 1996).

The gut, test, and lantern indices were identical in the light diet-rearing treatments. Therefore, neither the food type nor the environmental parameters seemed to affect, over a 6-month period, the partitioning of weight into these various body components. It has similarly been observed in other species that food quality does not have any effect on the size of the gut (Klinger et al. 1994 1995). The different body compartments in the reared sea urchins contain storage material in the form of lipids and carbohydrates (Fernandez 1997).

CONCLUSION

The results obtained reveal that the mixed formulated (vegetable and animal meal) and animal feeds (animal meal) allow for a higher somatic growth than do the vegetable feed types (natural food source made up of *Cymodocea nodosa* or formulated feed). In addition, observed growth was similar to or higher than that calculated from other studies on this species. The formulated feed was capable of inducing a higher growth rate than was seen with natural food sources.

The growth observed under rearing conditions exhibited distinct seasonal and monthly variations. These seem to be mostly attributable to water temperature (with a slower growth when temperatures were either very low or very high) but also to the organism's reproductive cycle (in particular, growth was very low during spawning events). Those tanks with a regulated temperature did not generate satisfactory results. Indeed, the semiclosed circuit used, which is without water renewal, produces a water quality that is insufficient to maximize growth. In particular, the level of nitrites were often high, which increases the sea urchins energy expenditure. Future experiments at a regulated temperature will need to include a water purifying system in order to generate irreproachable water quality.

The efficiency of formulated feeds is very important with respect to gonadal production. These feeds can generate a substantial increase in gonadal growth that is of far greater importance than that observed in the natural environment. This growth occurs even in small-sized individuals for which the gonadal index can reach up to 25% of the sea urchin's total mass. It is, therefore, possible that sea urchins smaller than those usually eaten may be able to generate a gonadal production equivalent to that produced by larger individuals. It would, therefore, be possible to reduce rearing time required before commercialization.

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FEEDING RATE, ABSORPTION EFFICIENCIES, GROWTH, AND ENHANCEMENT OF GONAD PRODUCTION IN THE NEW ZEALAND SEA URCHIN *EVECHINUS CHLOROTICUS* VALENCIENNES (ECHINOIDEA: ECHINOMETRIDAE) FED PREPARED AND NATURAL DIETS

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ABSTRACT Small, (30–40-mm diameter) medium, (50–60-mm diameter) and large (70–80-mm diameter) *Evechinus chloroticus* Valenciennes collected from Doubtful Sound on the west coast of the South Island of New Zealand were held in individual containers with running seawater and fed prepared and natural diets during the austral autumn, winter, and spring. At the beginning and end of each experimental period feeding rates, absorption efficiencies, growth, and gonad and gut indices were determined.

Feeding rates differed significantly between diets, urchins eating more of extruded pellets than agar-bound prepared feed or natural algal food. Feeding rate showed a clear seasonal trend directly correlated with water temperature. Absorption was quite variable but did not differ significantly between diets or with urchin size. Absorption was significantly lower in December than in other months. Gonad indices were higher in urchins fed prepared feeds than algae; however, there were seasonal differences in which of four different prepared feeds produced the largest gonads. Small urchins developed gonads when fed in the laboratory, although this size class does not develop gonads in the wild. This suggests sexual maturity may depend upon nutritional state as well as size. Prepared feeds tended to produce white- or cream-colored gonads unsuitable for sale; whereas, urchins fed algae generally formed yellow/orange gonads. Gut indices were similar in the three size classes of urchins and were generally higher in urchins fed prepared feeds. Small urchins grew proportionally more than medium or large sizes. Growth was faster on algae than prepared feeds.

Although prepared feeds can enhance gonad production in *Evechinus chloroticus* the composition will need to be appropriate to achieve acceptable yellow/orange gonad color and better somatic growth.

KEY WORDS: sea urchin, *Evechinus chloroticus*, enhancement, gonad, growth, feeding rate, absorption

INTRODUCTION

The New Zealand Echinometrid *Evechinus chloroticus* (known locally as *Kina*) is common and widely distributed on rocky reefs to depths of 5 to 10 m around the coast and outlying islands, although they can occur at greater depths in some areas. Gametogenesis (gonad growth) commences in the winter (April to May), and ripe gonads are present from October to November, with spawning from January to March (Walker 1982, McShane et al. 1996), generally being earlier in more northern areas of the country. The roe (gonads) has been harvested on a small scale by indigenous Maori since before the arrival of Europeans, and small quantities of roe have been sold onto the domestic fish market for many years for consumption by Maori (Hartstone 1984). Attempts have been made more recently to develop larger-scale fisheries for export to markets in Southeast Asia, which is already supplied by many countries, particularly the east and west coasts of the United States and South America. However, there has been a major problem developing such fisheries for *E. chloroticus* because of an apparent bitter taste, variable and low recovery of roe, and generally poor color of the roe (McShane et al. 1994, McShane 1996). For example, in November 1992, an experimental fishery for sea urchins was started in Dusky Sound on the southern west coast of the South Island, Fiordland. One thousand tons of quota for urchins was sold to five joint venture companies, but only a small

proportion of the original allocated quota was fished, and little of this was marketed because of problems with the roe quality. Approximately 8 to 8.5% of the wet weight of urchins caught was recovered as roe and a maximum of 20% of this was successfully marketed in Southeast Asia. That means a total of less than 2% of the weight of urchins collected was actually sold. The future viability of fisheries for *E. chloroticus* is clearly in doubt with such catch returns, although the stocks of harvestable urchins seem very large.

Studies have shown that sea urchins produce normal gonads when fed prepared feeds (Levin and Naidenko 1987, Klinger et al. 1997, Lawrence et al. 1992, Walker and Lesser 1996). Furthermore, Lawrence et al. (1997) obtained much better gonad production in the sea urchin *Loxechinus albus* using prepared feeds as compared to natural algal diets. The recent use of such prepared feeds for sea urchins has raised the possibility of improving the quality of gonads of *E. chloroticus* with such diets. This study reports the results of experiments in which *E. chloroticus* were fed different prepared feeds and absorption, feeding rates, growth and the quantity and quality of gonads determined at different times of the year.

MATERIALS AND METHODS

Three experiments using the same experimental design and spanning the main period for gonad development, were conducted

TABLE 1.
Diet treatments and dates of experiments.

	Dates	Diet Treatment	Sizes of Urchins	No. of Urchins (All Treatments in Each Exp.)
Experiment 1	February 1–April 30, 1994	a) Soybean meal mix b) Soybean/fishmeal c) Algae d) Starved	Small Medium Large	15
Experiment 2	May 24–Sept. 5	a) Soybean meal mix b) Soybean/fishmeal c) Algae d) Starved e) Pellets 4002 f) Pellets 4004	Small Medium Large	10
Experiment 3	October 3–December 1994	a) Soybean meal mix b) Soybean/fishmeal c) Algae d) Starved e) Pellets 4002 f) Pellets 4004	Small Medium Large	10

over a 10-month period from February to December 1994 at the Portobello Marine Laboratory. The first experimental period, February to May, was immediately after the breeding season, the second, June to September, was during the main period of gonad growth and the third, October to December, during late gametogenesis through to the time of early spawning. During these experiments, sea urchins of three size classes: (1) small, sexually immature (30 to 40-mm diameter); (2) medium, just reaching sexual maturity (50 to 60-mm diameter); and (3) large sexually mature (70 to 80-mm diameter) were fed different diets (Table 1). The feeds prepared from meal with agar as a binder are those used by Klinger et al. (1994), and the composition is given in Table 2. Two formulations of extruded diets in the form of pellets were used (Table 3). One (4002) contained high concentrations of corn grain and wheat middens and kelp, which were absent in the other (4004). Feed 4004 contained wheat starch and cellulose, which

were absent in the other (4002). Feed 4004 also contained more than twofold the concentrations of menhaden and soybeans. The algal diet was a mixture of, *Macrocystis pyrifera* (Linnaeus) and *Ulva lactuca* Linnaeus freshly collected from Otago Harbour, both natural foods of *Evechinus chloroticus*. Feeds prepared with meal and algal feeds were used for all three experiments. The extruded feeds were used for the second and third experiments. *Evechinus chloroticus* used for all three experiments were collected from Espinosa Point in Doubtful Sound, Fiordland. In the laboratory, urchins were placed in individual plastic containers with fitted lids. The size of the container varied with the size of the urchin, (small = 1,000 mL, medium = 2,000 mL, large = 4,000 mL). Each container was supplied with an individual supply of filtered seawater. The containers were arranged in a random block design in 6 large (1,440 × 740 × 200 mm deep) fiberglass tanks. Each tank received ambient light from nearby windows. Urchins were checked twice daily, and new food was supplied twice weekly so that animals fed *ad libitum*. Any particular food allocation not consumed in 3 days was removed, and fresh food was supplied.

TABLE 2.

Composition (in percentage) of prepared feeds using meals. The meals were mixed with agar (5 g meal: 4 g agar in 100 mL water).

Constituent	Soybean and Fish Meal	Soybean Meal
Fish meal	28.6	0.0
Soybean protein	23.2	44.5
Cellulose	5.9	5.9
Wheat starch	27.5	25.9
Menhaden oil	0.0	2.4
Cholesterol	0.5	0.5
Lecithin	0.9	0.9
Mineral mix	9.1	15.3
Vitamin mix ^a	2.6	2.6
Chromic oxide	2.0	2.0

^a 150 ppm thiamin, 100 ppm riboflavin, 400 ppm niacin, 400 ppm calcium pantothenate, 150 ppm pyridoxine hydrochloride, 20 ppm vitamin B-12, 80 ppm folic acid, 20 ppm biotin, 1,000 ppm inositol, 100 ppm PABA, 4,500 IU/kg vitamin A, 400 IU/kg vitamin E, 2,000 ppm choline, 20 ppm menadione, 4,000 IU/kg vitamin D₃, and 150 ppm vitamin C.

Analyses

At the beginning of each experiment, the diameter of each animal was measured with Vernier callipers to the nearest 0.01 mm. Ten urchins randomly selected in each size category were also dissected, and gonad and gut indices were determined as the wet weight of the organ expressed as a percentage of the total wet weight of the sea urchin (test and lantern). Prior to weighing, the gut was carefully washed to remove pellets. The sex of each urchin was determined by examining a smear of gonad tissue under a compound microscope or from histological sections of gonad tissue fixed in 10% formalin in seawater. At the end of the experiment, the diameter of each laboratory animal was measured, and the urchin was dissected and organ indices determined as before. A sample of 10 urchins in each size category was also collected from the field population, and the gut and gonad indices

determined as described above. Color of gonads was visually assessed.

Chromic oxide was incorporated as an insoluble component at a known ratio to the other components in the powdered soybean and fishmeal feed mixes. Analysis of chromic oxide in the feces was then used to determine absorption (McGinnis and Kasting 1964). Absorption was measured during the second week of feeding and in the last week before each experiment was terminated. Feeding rates were calculated over three consecutive 24-hour periods both at the beginning and at the end of the experiment by feeding the sea urchins preweighed food, collecting the uneaten food after 24 hours, blotting dry, and reweighing. For experiment 1, feeding rate was determined for all 15 urchins in each treatment. For experiments 2 and 3, feeding rate was determined for five randomly selected urchins in each treatment. For all diet treatments, three control containers were set up containing food but no urchin to determine if leaching of food occurred over a 24-hour period. Feeding rate is expressed as the average for 24 hours over the three periods.

Statistical Analysis of Data

Analysis of variance (ANOVA) was used to compare results of experimental treatments. Where appropriate, data were arcsine transformed before analysis and *post hoc* comparisons were made with Scheffe's F procedure. All statistical analyses were performed with the statistical package Statview 5.0.

RESULTS

Survival

Survival was 95% in experiments 1 and 2 and 96% in experiment 3. More animals died in the starved than fed treatments, but no more than two animals died in any one treatment for a particular size class.

Feeding Rate

There was no significant change in weight of agar blocks, pellets, or algae over a 24-hour period in containers that did not contain an urchin; that is, leaching of material from the food in the absence of an urchin did not occur. To compare the different diets, wet weights of the food consumed over 24 hours were converted

TABLE 3.

Composition (in percentage) of extruded feed pellets.

Constituent	4002	4004
Corn grain	32.0	0.0
Wheat middens	27.5	0.0
Wheat starch	0.0	23.3
Cellulose	0.0	5.8
Kelp	14.0	0.0
Menhaden	12.0	28.7
Soybeans	11.1	27.0
Phospholipid	1.0	1.0
Cholesterol	0.3	0.5
Minerals/vitamins ^a	2.0	11.7

^a 53.8 ppm thiamin, 83.6 ppm riboflavin, 442.3 ppm niacin, 118.1 ppm vitamin E, 23.0 ppm vitamin K, 112.5 vitamin C, 83.6 ppm riboflavin, and 46.5 ppm pyridoxin.

TABLE 4.

ANOVA of feeding rate of small, medium, and large *E. chloroticus* for experiments 1, 2, and 3 but excluding extruded diets (pellets 4002 and 4004) in the analysis.

Source	DF	Sum of Squares	Mean Square	F-Value	p-Value
Diet	3	0.25	0.083	19.385	<.0001*
Size	2	0.729	0.364	84.622	<.0001*
Time	5	0.562	0.112	26.107	<.0001*
Diet · size	6	0.035	0.006	1.367	.2259
Diet · time	15	0.187	0.012	2.89	.0002*
Size · time	10	0.138	0.014	3.214	.0005*
Diet · size · time	30	0.218	0.007	1.688	.0137*
Residual	511	2.2	0.004		

to dry weights for all diets. These were compared by a three-factor ANOVA, the factors being diet, urchin size, and time. Because experiments 2 and 3 included two additional diets (pellets 4002 and 4004), two separate analyses were performed. In the first (Table 4), feeding rates on alga, soybean and fishmeal diets only were analyzed for the three experiments. In the second (Table 5), all diets were included, but only feeding rates in experiments 2 and 3 were compared. Feeding rate is shown in Figure 1 as g dry weight of food eaten per day from February to December, 1994. There was considerable variation between individual urchins in each size and for each diet treatment, indicated by the wide standard errors in Figure 1. The results from the three consecutive experiments plotted through the year generally show a reduction in the daily amount consumed from February to a low in June and August and then a rise through spring and early summer. This is more marked in the prepared feeds than the algal diets. Winter reduction in feeding mirrors the change in water temperatures over this period (Fig. 1). There was also a difference in feeding rate between different sized individuals, large individuals >medium >small. ANOVA for both size and time show these differences in feeding rate to be significant ($p < .0001$).

There was also a significant interaction between size and time for both algae, soybean, and fishmeal diets ($p < .0005$, Table 4) and for experiments 2 and 3 ($p < .05$, Table 5) indicating that trends changed between experiments. There were no significant differences ($p > .05$) between the two pellet diets (4002 and 4004) or between the soybean and fishmeal diets. There were however

TABLE 5.

ANOVA of feeding rate of small, medium, and large *E. chloroticus* for all diets for experiments 2 and 3 only.

Source	DF	Sum of Squares	Mean Square	F-Value	p-Value
Diet	5	1.425	0.285	22.45	<.0001*
Size	2	0.839	0.419	33.027	<.0001*
Time	3	0.496	0.165	13.013	<.0001*
Diet · size	10	0.13	0.013	1.026	0.4214
Diet · time	15	0.437	0.029	2.293	0.0044*
Size · time	6	0.162	0.027	2.126	0.05*
Diet · size · time	30	0.451	0.015	1.185	0.2388
Residual	285	3.618	0.013		

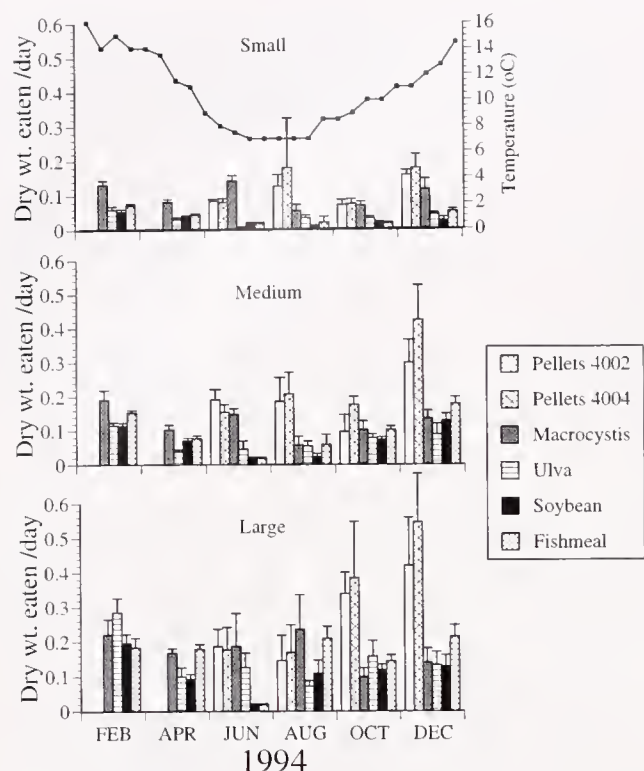


Figure 1. Feeding rates (g dry wt./day \pm SE) of small, medium, and large *E. chloroticus* at the beginning and end of the three experiments. Because different individuals were used in each experiment, datapoints for urchins on similar diets are not connected. Temperature ($^{\circ}$ C) as single measurements collected over the experimental period is also shown.

marked differences ($p < .0001$, Tables 4 and 5) in feeding rates among the major diet categories (extruded pellets > meal-agar > algae). From February to June greater amounts of *Macrocyctis pyrifera* were eaten by small and medium-sized urchins, with little difference between the other diets. During this period, large urchins showed considerable variation in the diet preferred initially eating more *Ulva lactuca* than fishmeal in April. When the pellet diets were introduced in June, these tended to be eaten more by all size classes through to the end of the study. This was particularly evident from August through to December, when the feeding rates on these feeds were frequently more than twice that of the other feeds and feed 4004 being eaten more than feed 4002 (Fig. 1). The significant interaction ($p = .0002$, Table 4) between diet and time reflects the variation over time between the diets eaten more by the different sized animals. However, during experiments 2 and 3, the rates of feeding were more consistent (diet \times size \times time interaction $p = .2388$, Table 5).

Absorption Efficiencies

Percentage absorption for the small, medium, and large animals fed on the fishmeal and soybean diets is shown in Figure 2 for experiments 1, 2, and 3. Absorption varied between diets and with time and was also quite variable between different individuals within an experimental treatment. The mean value for any particular treatment however was generally between 12 to 25%. A three-

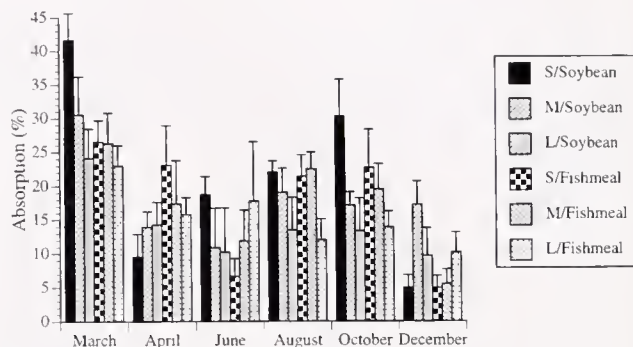


Figure 2. Absorption (% \pm SE) of soybean and fishmeal diets for the three experiments for small (S), medium (M), and large (L) *E. chloroticus*.

factor ANOVA of arcsine transformed data was used to compare absorption the factors being diet, urchin size, and time (Table 6). There were no significant differences ($p > .05$) in absorption between the two diets or between the three sizes of urchins, and there were no significant interactions ($p > .05$) between the various factors. There was, however, a significant difference between dates ($p = .0001$, Table 6). Scheffe's *post hoc* test showed the percentage absorption for March to be higher than for all other months ($p < .02$) and percentages absorption in October to be higher than December ($p = .0196$).

Gonad Indices

Gonad indices are shown in Figure 3. Single-factor ANOVA of arcsine-transformed indices was used to compare diet treatments for each experiment and *post hoc* comparisons were made with Scheffe's F procedure. ANOVA showed no significant difference ($p > .05$) between male and female gonad indices and for comparison of indices data from both sexes pooled.

The same general trends in gonad indices were shown for the three experiments and for the three urchin sizes. When gonads were developed in small urchins, they were never higher than 7%. In medium urchins, laboratory fed treatments varied from 5 to 15 and in large urchins ranged from 15 to 25%.

For all experiments and for all sizes of urchins, gonad indices of unfed animals were significantly lower ($p < .05$) than fed ur-

TABLE 6.

ANOVA of absorption efficiency of small, medium, and large *E. chloroticus* fed on the fishmeal and soybean diets from March until December 1994.

	DF	Sum of Squares	Mean Square	F-Value	p-Value
Date	5	6347.004	1269.401	14.622	<.0001
Diet	1	42.76	42.76	0.493	0.4842
Size	2	470.155	235.078	2.708	0.0708
Date \times diet	5	703.326	140.665	1.62	0.1599
Date \times size	10	1130.749	113.075	1.303	0.237
Diet \times size	2	130.585	65.292	0.752	0.4736
Date \times diet \times size	10	979.356	97.936	1.128	0.3472
Residual	118	10243.869	86.812		

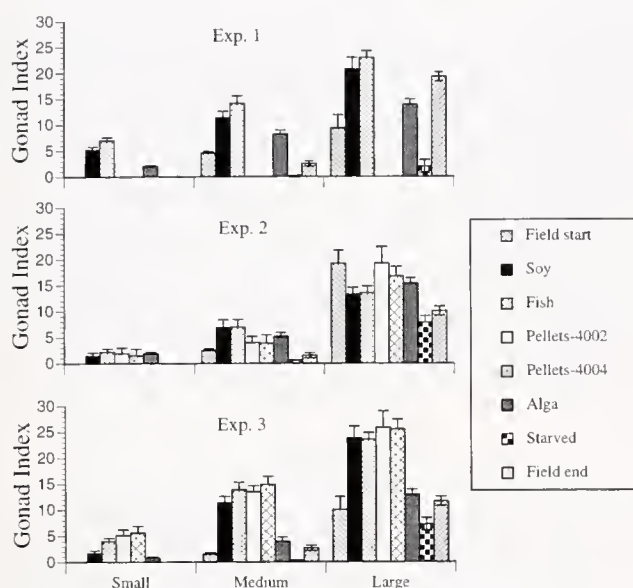


Figure 3. Gonad indices (\pm SE) of *E. chloroticus* fed different diets for experiment 1, experiment 2, and experiment 3.

chins. Extruded pellet diets gave higher mean indices than powdered agar diets or algae and of the four prepared diets soybeans were inferior to the others.

In experiment 1 (Fig. 3) during the postspawning period, gonads were produced in the smallest size class fed prepared diets or algae, although gonads were significantly larger ($p < .005$) on the two prepared diets than those fed algae. Small urchins collected from the field both at the start and end of the experiment did not develop gonads. In medium-sized urchins, gonad indices were significantly higher ($p < .0001$) using fishmeal than soybean or algae diets. All fed urchins had significantly larger ($p < .01$) gonads than urchins from the field. Gonads of large urchins fed soybean were significantly larger than those fed algae ($p < .005$); however, there were no other significant differences between diets. Gonads of large fed urchins were not significantly different ($p > .05$) to the gonads of urchins collected from the field at the end of the experiment.

In experiment 2 (Fig. 3), during the period of gonad growth, extruded pellets were included in the diet treatments. Gonad indices were generally lower for all size classes than in experiment 1. For small urchins, the gonad index was low and very similar in all laboratory fed treatments. For medium urchins, gonad indices were significantly higher ($p < .01$) in fed than field urchins collected at the end of the experiment, and there were no significant differences in gonad indices between any of the fed urchins ($p > .05$). Although Figure 3 shows clear differences in gonad indices, these were only significant ($p < .02$) between fed and starved urchins.

In experiment 3 (Fig. 3), the period of late gametogenesis, gonad indices were generally similar in small and medium-sized animals to those obtained in experiment 1; however, large urchins had the highest indices obtained during any of the experiments. In small, fed urchins, soybean diets produced significantly smaller indices ($p < .05$) than the other prepared diets, and indices were significantly higher on pellets ($p < .01$) than algae. In both medium and large urchins, there were no significant differences ($p > .05$) between any of the four prepared diets, and these diets gave sig-

nificantly higher indices ($p < .05$) than urchins fed algae or collected from the field at the end of the experiment.

For all three experiments, the gonads of urchins fed on all prepared diets, although large, were consistently very light in color, ranging from white to cream. The gonads of animals fed kelp were much more yellow to orange.

Gut Indices

Gut indices are shown in Figure 4. Single-factor ANOVA of arcsine-transformed indices was used to compare diet treatments. Gut indices for fed experimental treatments ranged from 7 to 10 but did not vary greatly between different sized urchins or between the three different experiments. The only significant difference between fed diets occurred in experiment 2, where small urchins fed soybean and fishmeal had significantly higher ($p < .05$, Scheffé's *post hoc* test) gut indices than all other fed treatments. For all sizes and in all experiments, fed urchins had significantly larger ($p < .0001$) gut indices than starved treatments. Starved urchins also generally had significantly smaller gut indices than field animals. For small and medium urchins, these were different at the $p < .0001$ level of significance, for large urchins in experiments 1 and 2 at the $p < .05$ level of significance. In experiment 3, there was little difference between starved and field animals at the end of the experiment.

Urchins collected from the field, however, did show large differences in gut indices to other treatments. At the beginning of experiment 1 (Fig. 4), gut indices of small, medium, and large field animals were high but were significantly lower ($p < .0001$) at the end of the experiment. In experiment 2 (Fig. 4), gut indices were low at the start of the experiment, although these differences were not significant ($p > .05$). In experiment 3 (Fig. 4), as in experiment 1, indices were higher at the start than the end of the

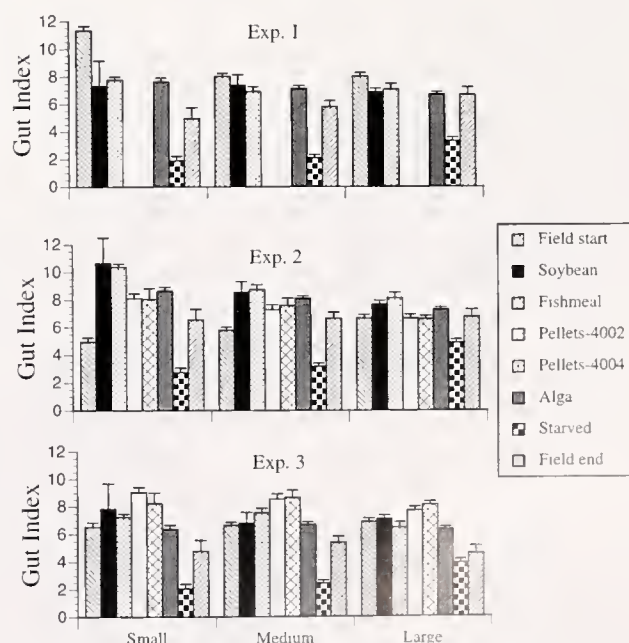


Figure 4. Gut indices (\pm SE) for small, medium, and large *E. chloroticus* fed different diets for experiment 1, experiment 2, and experiment 3.

experiment; however, the difference was only significant ($p < .025$) for large urchins.

Growth

Growth of urchins in the three experiments is shown in Figure 5 as percentage increase in diameter. Percentage increase in diameter for the different diets was compared by single-factor ANOVA for arcsine-transformed data.

In experiment 1, similar changes in diameter occurred for both small and medium urchins (Fig. 5). However, the percentage increase was much greater in smaller animals. Small urchins fed alga grew significantly more ($p > .0001$) than urchins fed soybean or fishmeal. Medium urchins fed algae grew larger than those fed fishmeal ($p > .03$). For all sizes, starved urchins decreased significantly in diameter ($p > .0001$). Very little growth occurred in large urchins, and there were no significant differences in diameter.

In experiment 2, sizes of urchins used in fishmeal and soybean diets were not recorded at the beginning of the experiment, so the only comparisons made were between urchins fed pellets and algae (Fig. 5). There was considerable variation between individuals, indicated by the wide standard deviations shown in Figure 5. In small and medium urchins, the greatest increase in diameter occurred in urchins fed algae, although the differences were not

statistically significant ($p < .05$). Unfed urchins grew very little and were significantly smaller than fed animals ($p > .03$). Large fed urchins also grew little and there were no significant differences between fed diets ($p < .05$). Starved urchins were significantly smaller ($p > .003$) than fed animals at the end of the experiment.

In experiment 3, although the diameters were different in small urchins fed the five different diets at the end of the experiment, the only significant differences were between the fishmeal and 4002 diets ($p < .005$) and the fed and starved urchins ($p < .005$) (Fig. 5). Medium-sized urchins grew less, and large urchins grew little. In these latter size classes, the only statistical difference at the end of the experiment was between starved and fed treatments ($p < .03$).

DISCUSSION

Feeding on all diets decreased over the cooler winter months (experiment 2) and increased again during the spring and early summer (experiment 3). A similar seasonal change in feeding has been noted by Klinger et al. (1986) and Klinger et al. (1997) for *Lytechinus variegatus* and for *Strongylocentrotus droebachiensis*, respectively. There was also a significant increase in feeding rate in larger urchins. Clearly, the metabolic needs of larger individuals will demand a greater intake of nutrients. The quantity of each of the two different types of prepared feeds eaten did not differ significantly, although pellets 4002 were eaten in greater quantities by all three size classes from August through to December, and there was little difference between the soybean and fishmeal diets. Klinger et al. (1994) found no difference in feeding of *Lytechinus variegatus* fed similar soybean and fishmeal diets. However Klinger et al. (1997) found *S. droebachiensis* consumed less of an extruded pellet diet containing kelp than a similar composition with no kelp. Similarly, Lawrence et al. (1997) and McBride et al. (1997) found that *Loxechinus albus* and *Strongylocentrotus franciscanus* consumed less extruded feed than algae. We found that *Evechinus chloroticus* eat more of all prepared feeds than seaweeds, the natural diet of the animals.

The composition of agar bound and extruded diets used in several previous studies were very similar to those used in the present study, and the summer feeding rates found in these studies are compared in Table 7 to the early summer (December) feeding rates of similar sized *Evechinus chloroticus*. No previous studies have been done on species as large as the 80 mm (large) *E. chloroticus* used in the present study; however, both small (30–40 mm) and medium (50–60 mm) diameter urchins from different families show very similar feeding rates when fed prepared feeds or when fed algal diets, suggesting that metabolic requirements of echinoids are much the same for unrelated species of equivalent sizes.

Absorption efficiencies determined by the incorporation of an insoluble marker in the feed were only able to be measured for the two prepared feeds that used agar as a binder. Although there was considerable variation within each diet treatment and size category of urchin used, the mean absorption efficiency was close to 20 to 25%, and there was little difference between different sized urchins or between the two diets. In December, absorption was significantly lower than during all other months. There is no obvious reason why lower absorption should occur during this period, although feeding rates were also higher at this time. It is possible that higher feeding rates force food through the gut at a faster rate allowing less time for absorption. Measurement of gut residence

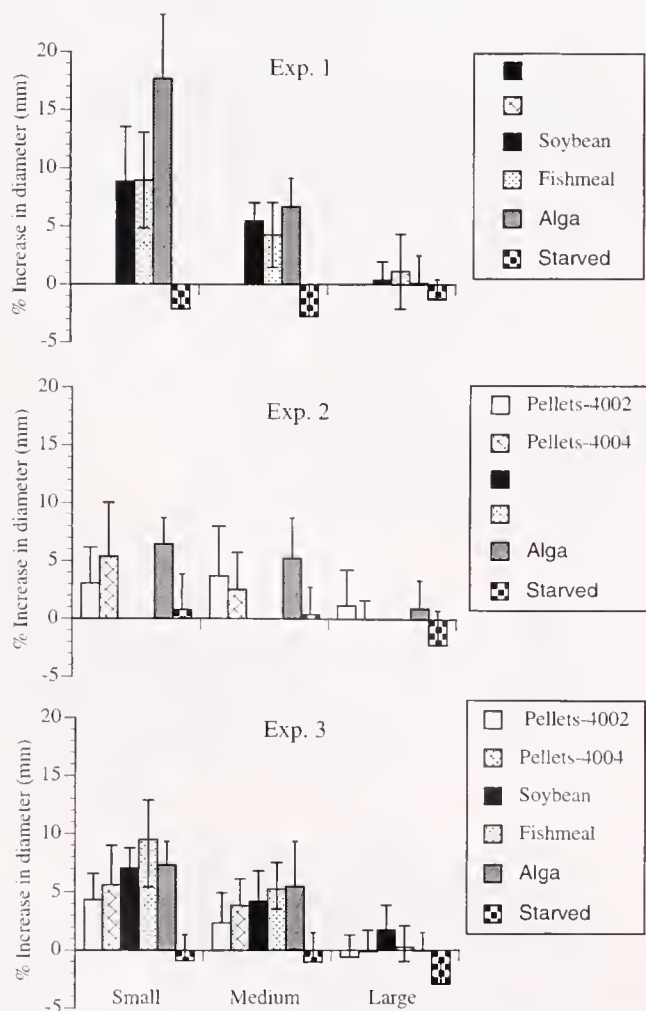


Figure 5. Percentage increase in diameter (\pm SD) of small, medium, and large *E. chloroticus* fed different diets for experiment 1, 2, and 3.

TABLE 7.
Feeding rate (g wet food/day) in the laboratory for a range of species determined over summer.

Author	Species	Diam (mm)	Wet wt (g)	Soybean	Fishmeal	Extruded 1	Extruded 2	Macrocystis	Ulva
Lawrence et al. 1997	<i>Loxechinus albus</i>		80			0.36	0.46	2.1	0.78
Klinger et al. 1997	<i>Strongylocentrotus droebachiensis</i>	47	50			0.63	0.44		
Klinger et al. 1994	<i>Lytechinus variegatus</i>	51	63	3.14	3.39				
Lawrence et al. 1988	<i>Paracentrotus lividus</i>		50	2	2.3				
Lawrence et al. 1989	<i>Paracentrotus lividus</i>	41			Approx. 2.0				
This study	<i>Evechinus chloroticus</i>	60	80	2.66	3.55	0.341	0.485	1.45	0.55
		37	26	0.6	1.2	0.18	0.2	0.81	0.19

time of ingested material would address this question. *E. chloroticus*, therefore, is slightly less efficient in absorbing nutrients than *Lytechinus variegatus* (mean absorption 32%, Klinger et al. 1994) but was very similar to *Paracentrotus lividus*, (20%, Lawrence et al. 1989), both these latter studies using almost identical diet formulations to the present study.

Unfed urchins showed no increase in gonad indices, and it is clear that in starved animals, allocation of energy to maintenance comes before reproduction. In fed animals, gonad indices generally increased over the course of the experiment; however, there were clear differences between experiments, diets, and with urchin size. Experiment 1 was conducted during the postspawning period, when most field populations should have spawned, and gonads would have regressed. However, the gonad indices of both medium and large animals were high in both laboratory animals and those collected from the field. Although there was little change in gonad size, histological examination of the gonad tissue showed that gonads in February were mostly composed of unspawned gametes; however, by May, gonads were made up of nutritive phagocytes and relict gametes. From May to September (experiment 2), the gonads of field populations decreased in size, with little change over the next 3 months. This is surprising, because during this period of the year, gonad indices could be expected to be increasing rapidly prior to December to January spawning. The decrease during 1994 may be an anomaly for that year. Annual variation in *E. chloroticus* gonad indices within and between populations in Doubtful Sound was also noted by Lamare (1997) and is probably common throughout New Zealand.

For all three experiments, small, medium, and large urchins fed both extruded and agar-bound artificial diets produced significantly larger gonads than urchins collected from the field or those fed algae. Lawrence et al. (1997) also found that larger gonads were produced by *Loxechinus albus* fed extruded diets than algae. Medium and large urchins consistently eat more of the two extruded diets than the agar-bound diets, but there was little difference between the gonad indices of urchins fed these different artificial diets.

Although the three experiments were conducted over different seasons, large gonads were developed in all three experiments, indicating that it is possible to use prepared feeds to produce gonads throughout the year, a similar result to that obtained by Lawrence et al. (1992) and Klinger et al. (1997). There were some seasonal differences in production in *E. chloroticus*, however, because gonads produced during the winter, when feeding rates were lower, were smaller than those formed during late summer or

spring. Lawrence et al. (1992) reported precocious development of gonads in small *Paracentrotus lividus* but did not examine the gonads. It is significant that small *Evechinus chloroticus* fed both prepared feeds and algae developed gonads at the completion of all three experiments, when urchins of this size collected from the field at any time of the year never contain gonads. When examined by histology, much of the gonad was composed of nutritive phagocytes. However, both oocytes and spermatocytes were found, confirming precocious gonad formation. This suggests that if surplus nutrients are available to those needed for maintenance and growth, they are allocated to gonad production, even in very small animals; that is, sexual maturity is determined by the availability of nutrients, not urchin size.

Urchins fed prepared diets, both meal and extruded, were consistently of a light cream to yellow color. Commercial markets require yellow or orange roe, suggesting that the feed tested here would be unsuitable for gonad production for mariculture.

Sea urchins may use the gut to store excess nutrients, which are later available for such metabolic needs as growth and production of gonads (Lawrence et al. 1966). Therefore the weight of the gut expressed as a percentage of the total dry weight (test and lantern), the gut index, gives a measure of the nutritional condition of the animal. There is less variation between gut than gonad indices of different sized urchins, indicating the gut is probably used for storage of excess nutrients throughout life, which probably does not change as urchins reach sexual maturity, providing nutrient levels remain high. Neither were there marked seasonal trends in indices in laboratory treatments, although urchins fed soybean and fishmeal had higher gut indices in experiment 2, and those fed extruded pellets diets had higher gut indices than those fed soybean or fishmeal feeds in experiment 3. The most obvious differences, shown in all three experiments, were between fed urchins and those collected from the field at the beginning and end of each experiment. In experiment 1, the gut indices were much higher in field populations at the beginning than end of the experiment, perhaps suggesting that high food levels were available over summer and that, during this period, nutrients were not required for gametogenesis. In experiments 2 and 3, conducted over a period when gametogenesis in the wild would be occurring, gut indices were lower in field than in laboratory fed urchins both before and at the end of the experiment, suggesting that nutrient availability was limited over these seasons.

There were marked differences in somatic growth between sizes, seasons, and diet. In all experiments, small urchins grew proportionally more than medium individuals, and large animals

grew very little. Starved individuals shrank. During autumn (experiments 1, soybean and fishmeal) and winter (experiment 2, extruded pellets), growth was better on algae than on prepared feeds; whereas, in spring (experiment 3), there was little difference. Klinger et al. (1997) obtained better growth of *Strongylocentrotus droebachiensis* on extruded diets containing kelp than feed without kelp. These observations suggest that the prepared diet formulations used in the present study may lack some essential components necessary to support maximal growth, particularly growth of the calcite test, although some growth is clearly possible. If prepared feeds are to be used in aquaculture of sea urchins

for growth in addition to increasing gonad volume, then further refinement of their composition is necessary.

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GONAD PRODUCTION IN THE SEA URCHIN *LYTECHINUS VARIEGATUS* (LAMARCK) FED PREPARED DIETS

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ABSTRACT Because management of the fisheries worldwide has not been successful, intensive efforts are being developed for aquaculture to meet the demand for sea urchins. The Gulf of Mexico does not have an established fishery for sea urchins, but one species shows great potential for a fishery and for aquaculture. *Lytechinus variegatus* exhibits rapid growth, and individuals reach gonadal maturity in less than 1 year. We measured roe production in adults fed two formulated diets for a period of 10 weeks in the laboratory. Gonads increased in size in individuals fed either diet, although roe production was less than the maximal roe production observed in natural populations. Gonad color varied with diet, which was attributed to differences in the primary ingredients of each feed. Proximate composition of the gonads indicated that diet had no effect on carbohydrate and lipid levels, however, protein levels increased with both diets.

INTRODUCTION

Worldwide, populations of sea urchins are declining in regions with developed fisheries (Keesing and Hall this volume). It is evident that land-based aquaculture will be necessary for timely development and management of the industry. Sea-urchin fisheries already exist on the Pacific and Atlantic coasts of North America (Sloan 1985), leaving the Gulf of Mexico as the last major body of water bordering the United States lacking a developed sea urchin fishery. Although the development of a fishery in the Gulf of Mexico has potential, many fundamental questions concerning the extent of the fishery, habitat alteration, and long term ecological impacts would require further assessment to avoid the problems that have plagued other sea urchin fisheries. However, technologies currently being developed in aquaculture may provide a solution for sustainable production of high quality roe.

Lytechinus variegatus (Lamarck) is common in the shallow waters of the Gulf of Mexico (Serafy 1979). Populations reaching 250 m² (Moore et al. 1963) have been found from southern Florida to Alabama, Texas, and Mexico (S. Hill pers. obs., Ernest and Blake 1981, Hopkins, pers. comm., Camp et al. 1973, Beddingfield 1997, Watts pers. obs., Valentine and Heard pers. comm., Pomory 1990, Caso-Muñoz et al. 1994) as well as in the Florida middle grounds (Hopkins et al. 1977). These reports suggest that large populations of sea urchins exist in shallow waters throughout the Gulf of Mexico.

Compared to other sea urchins currently fished in North America, *L. variegatus* has very rapid growth and early gonadal maturity, reaching 45 mm test diameter within 8 months of larval metamorphosis (Moore et al. 1963, Greenway 1977, Beddingfield 1997). The urchins can be held easily in aquaria and will consume natural (Moore and MacPherson 1965, Greenway 1977, Vadas et al. 1982) and extruded (Klinger et al. 1986; 1994) diets. These and other characteristics make *Lytechinus variegatus* an excellent candidate for aquaculture.

Understanding many aspects of the nutritional and energy requirements of *L. variegatus* will be essential before culture can occur successfully in a commercial operation. In the present study,

we report the production of gonads and their proximate composition in *L. variegatus* fed two prepared diets in the laboratory.

MATERIALS AND METHODS

Seventy-five adult *Lytechinus variegatus* (37–64-mm test diameter, 30–110 g wet weight) were collected in St. Andrews Bay, Florida (30°N; 85°W) in October, 1997 and transported to Birmingham, Alabama. Initially, fifteen individuals were blotted, weighed, and dissected. The stomach and intestine, collectively considered as the gut, and gonads were removed, blotted, and weighed, and gut and gonad indices were calculated (tissue wet weight divided by the total wet weight of the intact individual, multiplied by 100). Subsamples of the gonadal tissue were frozen and lyophilized for proximate analysis. The remaining individuals were maintained in aerated 80-L aquaria (8–10 individuals per aquaria) containing artificial seawater (32 ppt; Instant Ocean®) and equipped with undergravel (crushed dolomite) filters at 12:12 L:D and 20 to 22°C.

Thirty individuals were fed one of two prepared diet treatments (diet 1 or diet 2, Table 1) that were embedded in a 4% agar binder (25% prepared meal and 75% water) and proffered as a 1 cm³ block for 10 weeks. All individuals were fed daily *ad libitum*, and excess food was removed the next day, weighed, and the amount of food consumed by all individuals in each aquaria was recorded. Diet 1 was formulated by Dr. Addison Lawrence (Texas A&M University) specifically for sea urchins. Diet 2 was formulated by ourselves and is similar in composition to catfish diets, with the exception of the addition of alfalfa flour. At the end of 5 and 10 weeks, fifteen individuals were removed from each diet treatment and analyzed as described above.

Proximate analyses were performed on lyophilized gonads. Carbohydrate was determined by the method of Dubois et al. (1956). Lipid was determined by the method of Freeman et al. (1957) and protein was determined by the method of Lowry et al. (1951).

Significant differences among sexes, treatment groups, and times were determined for both gut and gonad indices using a

TABLE 1.

Composition of prepared meal. Constituents are given in percentages by weight. Feeds consisted of 25% prepared meal in a 4% agar matrix.

Constituent	Diet 1	Diet 2
Corn	24.89	10
Wheat midds	24.89	10
Soy flour	11.1	30
Alfalfa meal	—	29.1
Fish meal	12	10
Feather meal	—	10
Kelp meal	14	—
Sodium phosphate	1.33	—
Lecithin	1	—
Cholesterol	0.3	—
Ethoxyquin	0.2	—
Vitamin C	0.08	0.35
Vitamin/mineral premix	0.2	0.55
Potassium sorbate	0.3	—
Beta carotene	0.01	—
Fish oil (menhaden)	0.7	—
Glycerin	8	—
Phosphoric acid	1	—

Vitamin/mineral premix consisted of 150 ppm thiamin, 100 ppm riboflavin, 400 ppm niacin, 400 ppm calcium pantothenate, 150 ppm pyridoxine hydrochloride, 20 ppm vitamin B-12, 80 ppm folic acid, 20 ppm biotin, 1000 ppm inositol, 100 ppm PABA, 4500 IU/kg vitamin A, 400 IU/kg vitamin E, 4000 IU/kg vitamin D3, 150 ppm vitamin C. Minerals, magnesium sulfate (12.75 mg), zinc sulfate (0.55 mg), ferrous sulfate (2.5 mg), copper sulfate (0.0785 mg), ethylenediamine dihydriodide (0.0295 mg), cobalt sulfate (0.04 mg), sodium selenite (11.3 mg), and calcium carbonate (72.77 mg). The mineral concentrations listed are per kilogram of diet.

nested two-factor analysis of variance (ANOVA) (Minitab; normality and heteroscedacity were examined before analysis) followed by a two-sample Student's *t*-test. The relation of feeding time to gonad indices and of carbohydrate levels to the gonad index was determined by simple linear regression (Sigma Stat; significance determined at $p < .05$).

TABLE 2.

Average feeding rates of *Lytechinus variegatus* fed prepared or natural diets.

Diet	g wet weight individual ⁻¹ day ⁻¹	Reference
Diet 1	1.91	Present study
Diet 2	2.05	Present study
Prepared feed	4–8	Klinger et al. 1986
Prepared feed	3	Klinger et al. 1994
<i>Thalassia testudinum</i>	0.5–6.0	Moore and MacPherson 1965
Diet	g dry weight individual ⁻¹ day ⁻¹	Reference
Diet 1	0.44	Present study
Diet 2	0.47	Present study
<i>Thalassia testudinum</i>	0.128–0.133 (in situ)	Greenway 1977
<i>Thalassia testudinum</i>	0.06–0.1 (laboratory)	Greenway 1977
<i>Thalassia testudinum</i>	0.6	Vadas et al. 1982

TABLE 3.

Gut indices of *Lytechinus variegatus* fed for 5 or 10 weeks on diet 1 and diet 2. Values represent means (\pm SEM).

	Initial	5 Weeks	10 Weeks
	1.56 (0.11)	—	—
Diet 1	—	1.74 (0.15)	1.44 (0.08)
Diet 2	—	1.63 (0.01)	1.42 (0.13)

RESULTS

Lytechinus variegatus readily consumed the prepared diets in the agar binder. On average, individuals consumed 1.91 g and 2.05 g wet weight per day (0.44 and 0.47 g dry weight) for diet 1 and diet 2, respectively (Table 2).

The gut indices did not change significantly during the feeding period (Table 3). The gonad index increased with both diets, although the difference between diets was not significant (as determined by ANOVA) because of the large variation among individuals in each treatment at each sample time (Fig. 1). However, the gonads did trend significantly higher with feeding over time, particularly in those individuals fed diet 1 (linear regression, $p = .027$, $n = 45$) as compared to diet 2 (linear regression, $p = .082$, $n = 45$).

The levels of carbohydrate and lipid did not vary significantly with diet, although the level of carbohydrate trended to be greater with diet 1 than with diet 2 (Table 4). Levels ranged from 4.5 to 35% of dry weight and from 14.6 to 24% of dry weight for carbohydrates and lipids, respectively (Table 4). Linear regression analysis indicated that carbohydrate levels were significantly ($p < .001$) and positively correlated with gonad indices up to a gonad index value of two (Fig. 2). The level of protein increased significantly with feeding, the level was greater in diet 2 (Student's *t*-test; Table 4). Sex-specific differences in proximate composition were not observed.

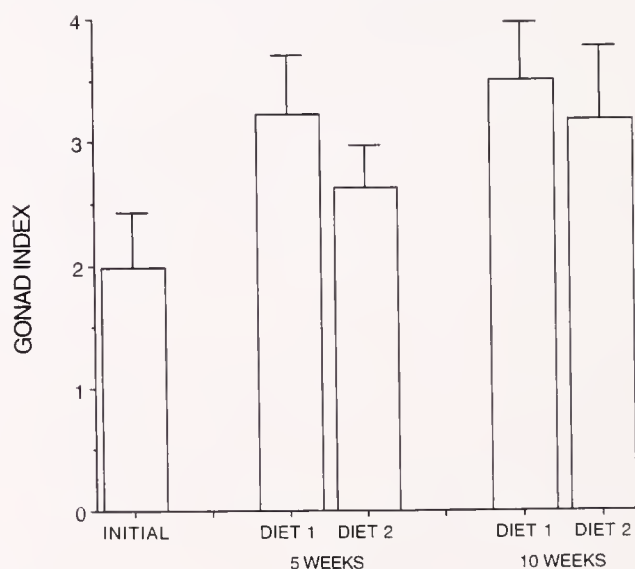


Figure 1. The gonad index of *Lytechinus variegatus* collected initially from the field and fed diet 1 or diet two for 5 or 10 weeks in the laboratory. Gonad indices of males and females were not significantly different and were combined ($n = 15$ for each mean; vertical lines represent SEM).

TABLE 4.

Proximate organic composition of the gonads of *Lytechinus variegatus* fed prepared feeds for 10 weeks.

Treatment	Carbohydrate	Lipid	Protein
Initial			
Males	20.6 (2.8)	18.9 (2.0)	14.2 (0.8)
Females	13.0 (4.9)	21.6 (1.3)	16.8 (1.5)
Diet 1 (10 weeks)			
Males	23.6 (2.2)	17.5 (0.8)	26.2 (0.8)*
Females	23.6 (3.5)	18.3 (0.4)	27.9 (1.3)*
Diet 2 (10 weeks)			
Males	18.0 (2.5)	17.4 (1.3)	30.1 (0.5)*
Females	16.5 (1.3)	18.7 (1.0)	30.2 (0.8)*

All values expressed as a percentage of dry weight (\pm SEM). Those values with an asterisk are significantly different (ANOVA, $p < .05$) from the initial values.

DISCUSSION

Lytechinus variegatus readily consumed the prepared feeds in laboratory aquaria. No differences in feeding rates were observed between diet treatments. Feeding rates in the present study were comparable with feeding rates reported previously for prepared feeds (Table 2). Although the values reported in the present study are lower than those reported by Klinger et al. (1986) and Klinger et al. (1994); that is 2 versus 3 to 6 g individual⁻¹ day⁻¹, respectively, the ingestion of dry matter was higher in the present study because of the greatly reduced water content of the proffered feed. Consequently, the sea urchins consumed less bulk, but ingested a higher amount of dry matter. In addition, the diets differed in composition among the various studies. Future studies examining feeding rates and other digestive characteristics should address the quantity and quality of the prepared (or natural) feed in terms of water content, nutrient content, energetic content, and, finally,

absorption and assimilation of the nutrients in order to make meaningful conclusions and comparisons.

The quantity and quality of the prepared feeds are fundamental in affecting production of roe. Sea urchins fed prepared feed in the laboratory often produce larger gonads than those in the field (Fernandez et al. 1995, Lawrence et al. 1997). In the present study, this was not the case. The maximal average gonad index (GI) after feeding *L. variegatus* for 10 weeks was less than 3.5, regardless of the quality of the feed. There was no significant difference between males and females. Beddingfield (1997) reported similar GI values in *L. variegatus* held for 7 months in the laboratory and fed natural diets. We should note that our study was conducted in the fall and completed several months before the period of maximal gonadal increase is observed in field populations in the spring. Field populations of *L. variegatus* can produce higher GI, ranging between 6 and 10 prior to spawning in the northern Gulf of Mexico (Beddingfield 1997) and between 9.5 and 12 along the coast of Brazil (Junquiera et al. 1997). Other studies have used different criteria for determining GI in *L. variegatus* (Ernest and Blake 1981, Moore et al. 1963) and maximal GI reported in those studies are not directly comparable.

Although there was a significant positive correlation between the GI and the amount of time the individuals were fed, the level of gonad production was less than anticipated. In earlier experiments, gonad production averaged 6 to 7 GI using the same feeds in *L. variegatus* collected in the early summer (Watts et al., unpub. data). Wasson et al. (1998) reported GI values >10 in individuals fed for 6 weeks, suggesting that there is a potential for the production of larger gonads in *L. variegatus*. Although seasonal influences may affect gonad production in the laboratory, we believe that other factors may have contributed to the lack of production. For example, water was exchanged frequently to maintain water quality in the presence of the high organic loads provided by both the feeds and the feces. However, feeding rates remained constant throughout the study and suggested no adverse affect of water quality on the sea urchins. The most probable cause of low gonad production may be related to protozoan populations. Most of the individuals sampled at 10 weeks had obvious loads of an unidentified ciliated protozoan that were observed (visualized using fresh gonadal squashes on microscope slides) feeding on gametes and other cells in the gonads. These commensals/parasites have been observed in the gut of *L. variegatus* collected from field populations (Berger 1964, Groliere et al. 1978, Groliere et al. 1980). If the protozoans are, indeed, parasitic, we suggest that the protozoans could influence resource utilization and, consequently, allocation to the gonads. Further studies into the impact of these protozoans on sea urchin physiology, both in the field and in culture, is warranted.

Significant levels of carbohydrate were found in both the testes and ovaries of *L. variegatus*. The range of levels reported in the present study indicates that carbohydrate levels can vary by almost an order of magnitude and that carbohydrate levels may reflect the nutritional well-being of the individual. This is particularly apparent in those individuals with GI values less than two. Much of the carbohydrate is presumed to be glycogen (based on the presence of Na₂SO₄-precipitated, anthrone-reactive material, Watts, unpub. data). Diet did not significantly (because of high variance among the individuals) affect carbohydrate levels, although individuals fed diet 1 had ca. 37% higher carbohydrate levels than those fed diet two. Diet 1 contained a high percentage of corn, and corn starch may ultimately serve as a source of the carbohydrate stored

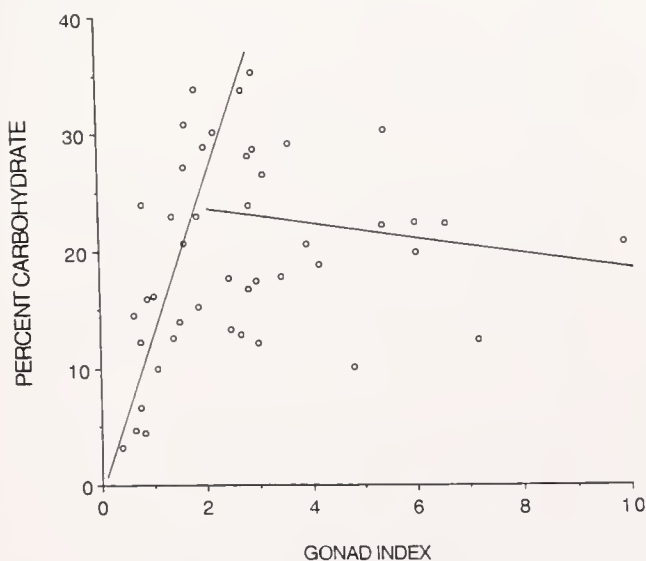


Figure 2. Relation of the percent of carbohydrate in the gonad to the gonad index. Lines represent linear regression analysis of carbohydrate to gonad index. A significant positive correlation exists when the gonad index is <2 ($p < .001$, $r = 0.77$, $n = 45$). No significant relation was observed at gonad indices >2 .

in the sea urchin gonad. Therefore, we suggest that carbohydrate levels can be altered by diet, and we hypothesize that alterations in carbohydrate levels may have an impact on taste, an important factor in sea urchin marketing.

Protein levels increased significantly with feeding and were highest in those individuals fed diet 2. Major sources of protein provided by diet 2 included fish meal, feather meal, and soy flour. The fate of protein assimilated into gonadal tissue has not been widely investigated and will require further studies. In contrast, the lack of significant changes in lipid levels suggests that lipids are not altered in response to diet quality, however, the total amount of lipid may be altered during growth of the gonadal tissue.

Prepared feeds can be optimized in terms of their composition and presentation to produce large gonads and to be nonpollutive and cost effective, as well. Both feeds in this study contained practical ingredients that are common in the aquaculture of sea urchins or other cultured species. However, the combination of practical ingredients that may result in an optimal feed has not been fully examined (Klinger et al. 1998). One of the most striking differences noted in the present study was the color of the gonads at the end of the study. Individuals fed diet 1 generally produced gonads that were light in coloration, somewhat translucent, varying from an ivory white to a pale yellow or orange. Those individuals fed diet 2 were generally darker in appearance, light brown, tan, or beige. We conclude that the primary ingredients in the feeds can influence gonadal color. This trait may be important

when developing sea urchin feeds which can enhance colors for specific markets. Similarly, Goebel and Barker (1998) found that some dietary ingredients (carotenoids) can influence gonad color.

In every major sea urchin fishery, populations have or are declining so that sustainable fisheries are unlikely. Inevitably, market demand will require the development of aquacultural practices that can supplement declining fisheries. To this end, environmentally and economically sound aquaculture practices can provide quality roe in sustainable yields. The potential for land-based intensive culture of sea urchins is being investigated (Birais and Le Gall 1986, Leighton 1995, de Jong-Westman et al. 1995, Fernandez et al. 1995, Fernandez 1996, McBride et al. 1997). We believe that *Lytechinus variegatus* has certain life history traits that indicate it is a very good candidate for aquaculture (Lawrence and Bazhin 1998). Future studies will consider optimization of roe production, the development of rearing techniques for larvae and juveniles, and the development of commercial-scale culture technologies.

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REVIEW OF HARVESTS AND STATUS OF WORLD SEA URCHIN FISHERIES POINTS TO OPPORTUNITIES FOR AQUACULTURE

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ABSTRACT It is more than a decade since there was an attempt to review sea urchin harvests and the status of sea urchin fisheries around the world. Much of this information resides in gray literature and unpublished reports, some of which are difficult to access. For many countries, only export figures are available, leaving uncertainty over total harvests and domestic consumption. Present world production is around 117,000 tonnes, most of which is imported into Japan, where local production has declined, and there are now attempts for this to be supported by stock enhancement methods. The world's major producers of sea urchins are the United States, Chile, and Japan. Catches of sea urchins in these and most other producing countries are declining or have reached their peak. Although several countries have underexploited sea urchin resources, these are insignificant in terms of world demand. The only countries with significant potential for expansion of sea urchin fisheries are perhaps the Russian Federation, Canada, and the two Koreas, but information from some of these regions is sparse, and there are a range of obstacles to sustainable and cost-effective exploitation. Given the general decline in fisheries in major producing countries and the continued strong demand for sea urchin roe, there are emerging opportunities for sea urchin aquaculture and roe enhancement in many countries.

KEY WORDS: Sea urchin, fishery, review, aquaculture, production, imports, exports

INTRODUCTION

It is more than a decade since there was an attempt to review sea urchin harvests and the status of sea urchin fisheries worldwide (see Sloan 1985). Although there is a wealth of published information on the biology and ecology of sea urchins around the world, much information on sea urchin fisheries resides in gray literature and unpublished reports, some of which is difficult to access.

There are a number of reasons why an up-to-date world situation report is pertinent at this time. Wild sea urchin stocks are a limited resource, and at the time of Sloan's (1985) review, many were in decline as a result of overharvesting. This has led to exploration and opening up of new fishing grounds, and some new fisheries have developed and declined even in the time since the review of Sloan (1985). Because most harvested sea urchins are destined for the Japanese markets (Hagen 1996), where demand continues to expand, the development of many of the world's sea urchin fisheries has been driven in response to changing economic circumstances and declines in other countries. Often the result is that jurisdictions are not well prepared to manage the new fisheries as they develop. Other countries are developing their fisheries in response to perceived market demands without a proper understanding of the situation in other countries. As fisheries worldwide have declined, sea urchin aquaculture has become highly topical (Parsons 1997) and is developing in those countries that have seen a decline in their wild stocks. Much of this development is occurring in the absence of information about the status of wild stock fisheries in a global sense, despite the economic feasibility of sea urchin aquaculture being largely dependent on sufficient wild stocks being no longer available. It is hoped this paper can contribute to a greater understanding of the over-all situation of sea urchin fisheries around the world and be an important reference for charting future trends and developments in sea urchin fisheries and aquaculture.

This paper attempts to record the different species of sea urchins being commercially exploited around the world, document the volume and value of production in each country, and provide

an overview of the status of as many of these fisheries. A range of information sources were used to assemble the review, including the traditional primary literature, gray literature, including workshop proceedings and internet sources, together with a number of personal contacts and communication.

Species of Exploited Sea Urchins

Table 1 provides an overview of the world's echinoid fisheries by country, region, and species. Where possible, the production in metric tonnes whole weight for 1995 is also given, but the table excludes harvests of sea urchins for ornamental purposes, about which there is little recorded information. Table 1 shows that there are at least 16 species of sea urchins harvested for food worldwide, with a total production of about 117,000 tonnes. The major species taken are those in the three big producing countries of Chile (*Loxechinus albus*), Japan (*Strongylocentrotus intermedius*, *S. nudus*), and the United States of America (*Strongylocentrotus franciscanus*, *S. droebachiensis*).

Patterns of Production in World Sea Urchin Fisheries

This section of the paper provides a description of the available information on trends in world sea urchin fisheries production by country or region followed by a brief account of the status of each fishery.

CHILE AND PERU

Chile is now the world's largest producer of sea urchins (*Loxechinus albus*), with landings of 54,609 tonnes in 1995 (FAO 1995). Production has increased rapidly since 1990, following declining catches in the late 1980s (Fig. 1). A review of the Chilean sea urchin fishery in 1992 pointed to overfishing and the impacts of the 1982 El Niño event as the cause of the then declining catches (Vasquez and Guisado 1992). Examination of the spatial trends in sea urchin harvest in Chile show this recent expansion in the fishery is a product of new grounds being opened up rather than through recovery of traditional fishing areas. Historically, sea urchin fishing was concentrated on the southern Chilean coast

TABLE 1.

World sea urchin landings in metric tonnes fresh weight by region, country, and species. Unless otherwise stated, source is 1995 figures from FAO (1995).

Region	Species	Country (State)	Production (t)
NW Atlantic	<i>Strongylocentrotus droebachiensis</i> (green)	USA (Maine) ^a	15,544
		USA (Massachusetts) ^a	78
		USA (New Hampshire) ^a	2
		Canada	2,850
NE Pacific	<i>Strongylocentrotus franciscanus</i> (red)	USA (California) ^b	10,086
		USA (Oregon) ^c	701
	<i>S. purpuratus</i> (purple)	USA (Washington) ^c	470
	<i>S. droebachiensis</i>	USA (Alaska) ^c	961
	<i>S. franciscanus</i>	Canada (British Columbia) ^c	6,328
	<i>S. droebachiensis</i>		
Japanese Islands	<i>S. franciscanus</i>	Mexico ^d	3,000
	<i>S. intermedius</i>	Japan (Hokkaido) ^e	5,163
	<i>S. nudus</i>	Japan (Pacific Ocean) ^e	3,923
	<i>Triplonectes gratilla</i>	Japan (Sea of Japan) ^e	807
	<i>Pseudocentrotus depressus</i>	Japan (East China Sea) ^e	3,455
	<i>S. pulcherrimus</i>	Japan (Seto Inland Sea) ^e	365
	<i>Anthocardis crassispina</i>	Japan (total)	13,735
	<i>Loxechinus albus</i>	Chile	54,609
South America		Peru	131
Korean Peninsula		North Korea	150
		South Korea	3,707
South East Asia	<i>Anthocardis crassispina</i>	Hong Kong	?
		Vietnam	?
		China	150
		Taiwan	63
		Philippines	466
South Pacific	<i>Heliocardis erythrogramma</i>	Australia ^f	93
	<i>Strongylocentrotus rogersii</i>		
	<i>Evechinus chloroticus</i>	New Zealand	804
	<i>Triplonectes gratilla</i>	Cook Island	25
Caribbean	<i>Triplonectes ventricosus</i>	Fiji	59
		Barbados	?
		Grenada	0
		Martinique	15
Northern Europe	<i>Strongylocentrotus droebachiensis</i>	Iceland ^g	923
	<i>S. intermedius</i>	Norway	?
	<i>S. nudus</i>	Russia	2,344
	<i>S. polyacanthus</i>		
Southern Europe	<i>Paracentrotus lividus</i>	Ireland ^g	10
		France	79
		Spain	?
		Portugal	?
		Malta	?
North Africa		Egypt	?
Total world sea urchin production			117,193

^a 1995 data from U.S. National Marine Fisheries Service, see Figure 3 for details; ^b 1995 data from Kalvass and Hendrix (1997), ^c 1995 data from PSMFC (1997), ^d approx. catch for 1994 from Kalvass and Hendrix (1997), ^e 1993 data from Sonu (1995), ^f Keesing unpublished, ^g FAO (1995) entries for these countries shown as *Echinus esculentus* are considered to be in error. *E. esculentus* is harvested in small quantities for the curio trade in the United Kingdom (Sloan 1985).

around 42°S to 43°S in what is known as region X. As these reefs became overexploited, fishing moved further south, most recently into region XII, where over half the total catch from Chile was taken in 1995 and 1996 (see Fig. 2, source Sernapesca, Chile via pers. comm. E. Bustos, Instituto de Fomento Pesquero, Puerto Montt, Chile). Catches in region XII have now also started to level off, and given the history of this and other sea urchin fisheries, it seems unlikely that catches can be sustained at this level. It is not

known whether further significant, but unexploited grounds, remain in Chile.

Information from Peru is limited to FAO production statistics for total echinoderms fished and Japanese import statistics. Total echinoderm production in Peru jumped to 131 tonnes in 1995 after 1994 landings was only 15 tonnes. Catches had fluctuated between 13 and 63 tonnes between 1990 and 1994 (FAO 1995). It is likely that much of this production is of sea urchins given Japanese

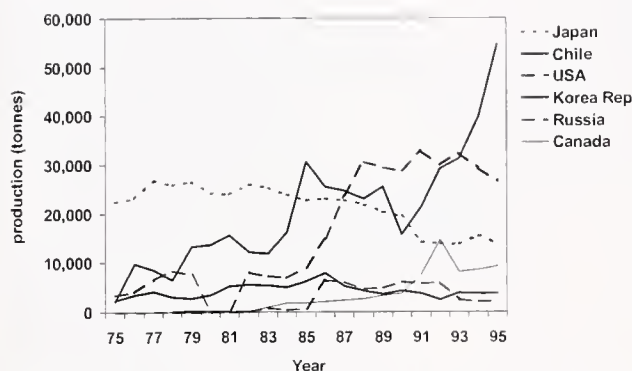


Figure 1. Twenty-year catch trend for the six major sea urchin producing countries: Japan, Chile, U.S.A., Korean Republic, Russian Federation, and Canada. Data from Sonu (1995), FAO (1995).

imports of Peruvian sea urchin products were 89 tonnes in 1996 (Table 2).

AUSTRALIA, NEW ZEALAND, AND SOUTH PACIFIC

Australia and New Zealand have largely unexploited sea urchin fisheries as a result of variable quality of product and marginal economics of harvest. Total Australian production in 1995 was just 92 tonnes of *Heliocidaris erythrogramma* and 1 tonne of *Strongylocentrotus rogersii* (Keesing unpublished). New Zealand produced 804 tonnes of *Evechinus chloroticus* in 1995 (FAO 1995). Although there exists potential for expansion of both the Australian (Sanderson 1996) and New Zealand (McShane and Naylor 1991, McShane et al. 1994) sea urchin fisheries, neither are likely to become major producers. FAO (1995) report small catches of sea urchins from Fiji and Cook Islands (Table 1) but little is known of these fisheries.

CANADA AND UNITED STATES OF AMERICA (NW ATLANTIC)

With the exception of the state of Maine, the eastern coast of the United States produces very little by way of sea urchin harvests (Table 1). Sea urchin (*Strongylocentrotus droebachiensis*) harvests in Maine increased rapidly from just 653 tonnes in 1987 to a peak of 19,115 tonnes in 1993. By 1995, catches had declined to less than 16,000 tonnes, with the most recent figures available (1997) showing a catch of 11,458 tonnes (Fig. 3). Preliminary figures for 1997 suggest a further decline to around 8,400 tonnes in 1997 (pers. comm., Ted Creaser, Maine Department of Marine Resources, W. Boothbay Harbor, Maine, U.S.A.). The pattern of

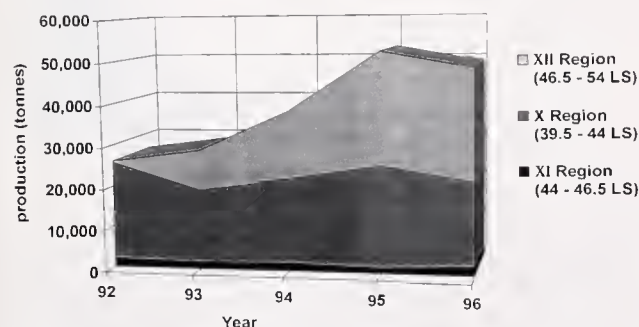


Figure 2. Five-year catch trend by fishing region for *Loxechinus albus* in Chile. See text for source of data.

rapid expansion and decline shown in the Maine catches is typical of a fishery developing on a largely unexploited resource of high density, accumulated age classes, and in the absence of significant regulation. Typically, as has occurred in Maine, the peaking and declining of catches in the face of continued expansion in effort stimulates restriction of access and license limitation. Measures introduced in Maine in 1995 to close some areas to fishing and restrict access have resulted in a reduction in the number of licences issued, particularly those using indiscriminate capture methods such as dragging, by about 50% to about 1,500 in 1996. It remains to be seen whether the most recent decline in catches points to a collapsing stock or whether the fishery has responded to the restrictions implemented and is now simply leveling off to a more sustainable level. Interest in aquaculture of *S. droebachiensis* in Maine has been partly attributed to concerns about overfishing of sea urchin stocks (Garrido and Barber 1998).

Canadian sea urchin catches in the NW Atlantic in 1995 were 2,850 tonnes (FAO 1995) having increased from 109 tonnes in 1990. Much of the catch comes from New Brunswick. Brian Burke (Canadian Centre for Fisheries Innovation, St Johns, Newfoundland, Canada) advises that the New Brunswick catch was 1,500 tonnes in 1996 to 1997. There are a number of suggestions that Canada may have the capacity to expand its east coast sea urchin production. As early as 1980 there were suggestions that Quebec could develop a sea urchin fishery based on the available resource (Kramer 1980). Whether the fishery can support the expansion required to make it a significant source among the world's harvests of sea urchins is not known. There is significant interest in aquaculture and roe enhancement of sea urchins in Canada (see various papers in Parsons 1997).

CANADA, UNITED STATES OF AMERICA, AND MEXICO (PACIFIC COAST)

The North American Pacific coast sea urchin fisheries (Fig. 4) are based principally on the red (*S. franciscanus*) and green (*S. droebachiensis*) sea urchins, although small amounts of purple sea urchins (*S. purpuratus*) are also caught. The combined catches from British Columbia in Canada and the U.S. states of Alaska, Washington, Oregon, and California make up a very important component of the world sea urchin catch producing over 18,000 tonnes in 1995 (Table 1) and, along with the Maine fishery, contributing over half of all sea urchin imports into Japan (Table 2). The largest of these fisheries is in California, where the 1996 catch was 9,100 tonnes (PSMFC 1997) down from 10,086 tonnes in 1995 (Kalvas and Hendrix 1997). Most of the California catch comes from the Southern Californian section of the fishery south of about 36°N. This fishery began in the early 1970s, and catches peaked at about 12 tonnes in 1990. Although catches in 1995 had declined to 7,940 tonnes, the fishery has been relative stable around the 8,000 to 10,000 ton mark since 1985 (Kalvas and Hendrix 1997). In contrast, the Northern California fishery did not develop until after 1984 and increased dramatically to 13,605 tonnes in 1988 before a collapse to just 2,148 tonnes in 1995. Kalvas and Hendrix (1997) provide an excellent review of the Californian sea urchin fishery and point to varying recruitment dynamics between the northern and southern areas of the fishery as partly explaining the comparative resilience of the southern fishery. They note that California, unlike most Pacific coast jurisdictions with sea urchin fisheries, has not moved to restrict catch through implementing quotas and that the current management

TABLE 2.
Summary of Japanese imports of sea urchins by country.

Country	Fresh/Live Imports (t)			1996 Total Imports (Quantity and Value)		
	1976	1986	1996	Tonnes	\$U.S. Millions	% of Total
U.S.A.	357.0	1243.0	3121.6	3209.3	144.54	50.92
Chile	85.0	263.0	458.8	1222.1	26.78	19.39
R Korea	985.0	1158.0	364.6	602.9	31.34	9.57
Canada	0.0	176.0	441.3	524.8	20.45	8.33
N Korea	266.0	239.0	7.9	262.2	2.73	4.16
China	12.0	225.0	209.3	239.9	9.35	3.81
Peru	0.0	0.0	79.7	89.5	2.45	1.42
Hong Kong	11.0	57.0	49.5	49.5	3.12	0.79
Iceland	0.0	0.0	19.1	35.7	1.04	0.57
Vietnam	0.0	0.0	0.0	21.8	0.18	0.35
Philippines	41.0	79.0	1.9	18.2	0.17	0.29
Mexico	82.0	98.0	0.0	17.8	0.40	0.28
Taiwan	35.0	61.0	0.0	4.1	0.02	0.07
Cook Is	0.0	0.0	0.0	2.5	0.01	0.04
Norway	0.0	0.0	1.0	1.0	0.03	0.02
Australia	0.0	3.0	0.7	0.7	0.04	0.01
Russia	0.0	0.0	0.0	0.4	0.01	0.01
Totals	1,874	3,602	4,756	6,302	243	100

Source: Sonu (1995).

methods of relying on effort controls and minimum sizes have been ineffective in preventing decline of sea urchin stocks.

The Washington state component of the Pacific coast catch in 1996 was 555 tonnes, of which about 350 tonnes was *S. franciscanus*, and the balance was *S. droebachiensis* (PSMFC 1997). This fishery has declined from over 4,200 tonnes in 1988 (Fig. 4). The fishery is now managed on the basis of total allowable catches for each species split into quotas for indigenous and nonindigenous fishers. The combined quotas of 650 tonnes were not caught in 1995 and 1996 mainly because indigenous fishers did not take their quota (PSMFC 1996, PSMFC 1997). Some areas of the Washington fishery remain closed to fishing as a result of sea otter predation reducing sea urchin biomass dramatically (PSMFC 1997).

The Oregon state fishery, which is mainly for *S. franciscanus*, has also declined dramatically from 4,200 tonnes in 1990 (Fig. 4) to just 701 tonnes in 1995 and 370 tonnes in 1996. Only 3% of the catch in 1995 was *S. purpuratus* (PSMFC 1996, PSMFC 1997). As a management measure, there is a strategy in place to reduce the number of harvesting permits in this fishery from the current level of 39 (PSMFC 1996, PSMFC 1997).

Alaska's green sea urchin (*S. droebachiensis*) fishery in the Kodiak Island (Gulf of Alaska) region produced 420 tonnes in 1996 (PSMFC 1997), down from 960 tonnes in 1995 (Table 1). Catches in this fishery have been variable for many years. Munk and MacIntosh (1993) suggested that discovery of new fishing grounds and highly variable recruitment are responsible for fluctuating catches from year to year in this region. More recently, a fishery based on the red sea urchin *S. franciscanus* has been developed in the far south of Alaska near Ketchikan and Prince of Wales Island, with a trial fishery producing about 1,300 tonnes in 1995 and 1996 (PSMFC 1997). A total allowable catch for this new fishery has been set at 2,090 tonnes (ADFG 1996).

The Canadian (British Columbia) component of the Pacific coast fisheries for *S. franciscanus* and *S. droebachiensis* was 6,400

tonnes in 1996 of which *S. franciscanus* made up over 6,200 tonnes (PSMFC 1997). The fishery expanded from 986 tonnes in 1983 to in excess of 14,000 tonnes in 1992 (PSMFC 1997) mainly through fishing new grounds in the northern part of B.C. (Thomas 1993). Since that time, catches have been constrained through the implementation of licence limits, total allowable catches, areal and seasonal closures, and a range of other management measures (Thomas 1993). Management tools in place for southern B.C. are area quotas, minimum and maximum size and seasonal closures, and rotational area openings and maximum allowable catches have been implemented in the northern part of B.C. to conserve stocks. The stability of catches since 1993 suggest the implementation of these management measures have been effective.

Sloan (1985) makes mention of a red sea urchin *S. franciscanus* fishery in Mexico, and Kalvass and Hendrix (1997) show Baja California harvest levels from 1987 to 1994, with catches fluctu-

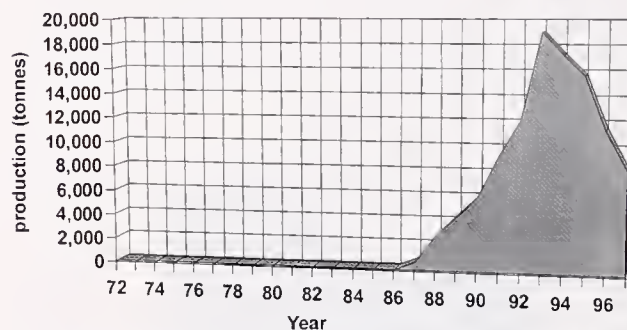


Figure 3. Recent sea urchin catch history for the state of Maine. Source: Annual Commercial Landings, Statistics, and Economics Division, National Marine Fisheries Service (NMFS). Available from National Oceanic and Atmospheric Administration (NOAA) Fisheries Headquarters, 1315 East-West Highway SSMC3, Silver Spring, MD 20910, U.S.A. Personal communication, Ted Creaser, Maine Department of Marine Resources, W. Boothbay Harbor, Maine 04575, U.S.A.

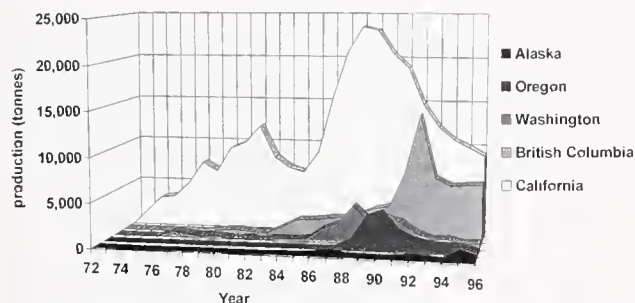


Figure 4. Recent sea urchin catch history for the Pacific coasts of the USA and Canada. Source: PSMFC (1997).

ating between 4,500 tonnes in 1989 to a low of 2,000 tonnes from 1991 to 1993. We could find no other reports to shed light on the status of the Mexican fishery.

JAPAN

Japan is the world's largest consumer of sea urchins and sea urchin products, importing 6,200 tonnes worth U.S. \$243 million in 1996. Up until 1984, Japan was also the world's largest producer (see Fig. 1). *Strongylocentrotus intermedius* and *S. nudus* make up about 80% of Japanese sea urchin harvests from Hokkaido and northern Honshu, and species fished in the southern regions of Japan are *S. pulcherrimus*, *Anthocidaris crassispina*, *Pseudocentrotus depressus*, and *Tripneustes gratilla* (Sonu 1995).

Annual production in Japan in 1995 was 13,735 tonnes (FAO 1995), which has declined from a peak of 27,528 tonnes in 1969 (Sonu 1995, Hagen 1996). Since that time, catches fluctuated between 27,000 and 23,000 tonnes right up to 1987, when it began to decline gradually falling below 20,000 in 1990. The biggest declines have been in the major producing areas of Hokkaido and the East China Sea (Fig. 5). These areas comprised 60 to 70% of all Japan catches since 1984, but since that time catches have fallen by 8,600 tonnes or 51%. Maps and descriptions of the eight fishing zones in Japan, and a breakdown of individual catches can be found in Saito (1992a) and Sonu (1995).

The cause of the decline in Japan sea urchin catches are not entirely understood and most texts (e.g., Saito 1992a, Sonu 1995, Hagen 1996) seem reluctant to suggest overfishing, but recruitment has clearly declined either as a result of overfishing, habitat degradation, disease, or most likely, a combination of these.

Declines in catches have lead to extensive restocking of cultured sea urchins in Japan. An excellent account of this is given in Saito (1992b). As yet, the benefits of restocking are not evident (Sonu 1995, Hagen 1996). Despite seedstock releases increasing from 8 million seed to 60 million seed between 1986 and 1992 (Saito 1992b, Hagen 1996), Japanese sea urchin catches continue to decline.

KOREAN PENINSULA

Little information is available on the North Korean sea urchin fishery other than the estimates that appear in the annual FAO statistics and Japanese import figures. Production in 1995 was estimated at 150 tonnes (FAO 1995), although Japanese import figures from as recently as 1994 show imports of 597 tonnes of frozen, dried, and salted sea urchin products from North Korea. There has been negligible imports of fresh sea urchin products from North Korea since 1987 (252 tonnes) with a clear shift to the preserved products (Sonu 1995).

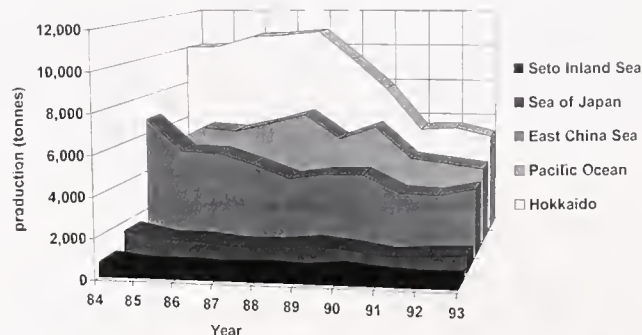


Figure 5. Annual production figures for Japan by region from 1984 to 1993. Data from Sonu (1995).

South Korean production of sea urchins was stable at 3,000 to 4,000 tonnes between 1990 and 1994 according to Voboril and Choi (1995). FAO (1995) figures show total production of echinoderms in 1995 was 3,707 tonnes having steadily declined from a peak of 7,785 tonnes in 1986. It can probably be concluded most of this was sea urchins given Japanese imports of sea urchin products in 1996 was 603 tonnes (Table 2).

Little information is available from either North or South Korea on the status of sea urchin stocks or whether any scope exists for expansion of these fisheries, and this cannot be ruled out.

CHINA, VIETNAM, AND THE PHILIPPINES

FAO (1995) statistics show modest harvests of sea urchins from China, Taiwan, Vietnam, and The Philippines (Table 1), although no other details are available.

THE CARIBBEAN AND THE GULF OF MEXICO

In 1986, Scheibling and Mladenov (1987) collected an oral history of the Barbados sea urchin fishery for *Tripneustes ventricosus*. At that time, they reported the fishery to have collapsed essentially to the point where urchins were rare or absent on traditional fishing grounds. Scheibling and Mladenov (1987) noted that no quantitative information was collected on landings at that time, but their research indicated that catches had declined from about 1,200 urchins per day to 140 per day over the decade leading up to their study. More recently Vermeer et al. (1994) reported that the annual harvest of sea urchins in Barbados was about 6,000,000 per annum with about 220 fisherman collecting sea urchins. Based on the assumption that the length of the fishing season and number of fishers was unchanged since Scheibling and Mladenov's study, catch rates would seem to have been reduced by a further 20% between the two studies. Sea urchins are fished in other parts of the Caribbean with FAO (1995) statistics showing small catches from Martinique and Grenada; however, the present status of production levels or stock abundance is not known.

Although there are no reports of commercial harvests of sea urchins from the Gulf of Mexico, Watts et al. (1998) report that significant sea urchin (*Lytechinus variegatus*) resources exist in this area offering the potential for commercial harvest and aquaculture.

NORTHERN EUROPE

Reid and Ovichinnikov (1995) reported that the latest reliable figures for Russia were from 1992 and 1993 when production of total echinoderms was 5,900 and 2,500 tonnes whole weight, respectively. These figures relate to the whole of the Russian Fed-

eration. FAO (1995) report catches of 2,344 tonnes in 1995. A breakdown of the catches in different geographic areas by species is difficult to obtain, because formal statistics are not collected in many areas. Table 3 has been collated from information kindly provided by Valery Levin (Kamchatka Research Institute of Fisheries and Oceanography, ul. Naberezhnaya 18, Petropavlovsk-Kamchatskii, Russia). Levin also cited a Russian publication by Sennikov and Matyushkin (1994) as reporting that there is about 7,100 tonnes of useable stock of *Strongylocentrotus droebachiensis* in the Barents Sea region near Murmansk. Reports by Gavrilova (1998a) (1998b) record a harvestable stock of 500 tonnes of *S. nudus* in the very south of Russia in Peter the Great Bay (zalev Petra Velikogo). Levin noted that current catches in the Barents Sea are negligible, and as with most other areas of Russia, much of the fluctuation and low catches is attributable to the marginal economics of exporting the product into Japan, particularly when the logistics of transport from remote areas is taken into account. Less than 0.4 tonnes of sea urchins was imported into Japan from Russia in 1996 (Table 2).

FAO (1995) show a record of sea urchin production in Norway and Iceland (Table 1), but no other details of these fisheries are known.

SOUTHERN EUROPE, THE MEDITERANEAN, AND NORTH AFRICA

Hagen (1996) reports that with an annual consumption of about 1,000 tonnes, that France is the second largest consumer nation of sea urchins after Japan. Production statistics show a 1995 harvest of only 79 tonnes of *Paracentrotus lividus*, although over 400 tonnes were harvested in 1992 (FAO 1995) and Sloan (1985) reports that *P. lividus* is harvested in southwest Ireland and exported to France. *P. lividus* is harvested on both the Brittany and Mediterranean coasts of France as well as in Spain, Portugal, Malta, Italy, and Egypt (see detailed discussion in Sloan 1985). Sloan (1985) reviews research work undertaken in the 1970s on the French Brittany coast fishery for *P. lividus*, which indicated overfishing had caused declined of stocks. Similar statements are made about the fishery in Mediterranean coasts but there is little recent information available (Sloan 1985). Moylan et al. (1998)

discuss the origins of the Irish fishery for *P. lividus*, noting that it developed quickly following the collapse of the Brittany coast fishery in France, peaking at just 350 tonnes in 1976, and since 1984, the fishery has not exceeded 105 tonnes. Again overfishing and unregulated access is regarded as having been responsible for the decline of the *P. lividus* fishery in Ireland (Byrne 1990, Moylan et al. 1998). The lack of recent reports on the status of sea urchin fisheries in the Mediterranean suggest they are at least fully exploited, if not overexploited, and most catches are destined for domestic markets. There have, however, been a range of aquaculture related studies on *P. lividus* in countries such as France and Ireland (e.g., Grosjean et al. 1998, Moylan et al. 1998, Spirlet et al. 1998) consistent with a worldwide trend to attempt to meet the continued demand for sea urchins through aquaculture following decline of wild capture fisheries. Recently, also, attempts have been made to establish a polyculture (with Atlantic Salmon) industry in Ireland based on enhancement of roe in *Psammonechinus miliaris* (Kelly 1998); however, there is no commercial fishery for the species at the present time in Ireland.

DISCUSSION

Sloan's (1985) review put world sea urchin harvest at 48,000 tonnes in 1982. Since that time, production has more than doubled, mainly through expansion of the Chilean, Canadian, Californian, and Maine sea urchin fisheries. Also since that time, sea urchin fisheries in California, Maine, and parts of Chile have built up and then begun to decline, or as in the case of Japan and Europe, continued to decline. Despite these trends, demand for sea urchin products remains high, with Japanese imports alone worth in excess of U.S. \$240 million.

The outlook for the world's sea urchins fisheries is not optimistic, given its recent history. With the United States providing over 50% of Japanese imports of sea urchins, declines in the Californian and Maine catches are likely to have a significant impact on the world market for sea urchins. It is not known how long the world's largest producer, Chile, can maintain such high levels of catch. Eventually, as newly discovered stocks are fished down, catches will inevitably fall and either stabilize or decline. Although the potential still exists for some expansion in sea urchin harvests in northern Europe, Canada, Russian Federation, and Korea, it

TABLE 3.
Summary of available information on recent catches (t) from areas of the Russian Federation.

Year	Region				
	Kamchatka	Sakhalin and South Kuril Islands	Japan Sea	Japan Sea	Barents Sea (Murmansk)
	Species				
	<i>S. polyacanthus</i>	<i>S. intermedius</i>	<i>S. intermedius</i>	<i>S. nudus</i>	<i>S. droebachiensis</i>
1992	3		1,066		
1993	60		1,373		130
1994	47				
1995	56				
1996	47		1,205		
1997	1		1,115		
		150 ^a		10–12 ^a	0 ^a

Source: various compiled by Valery Levin, Kamchatka Research Institute of Fisheries and Oceanography, ul. Naberezhnaya 18, Petropavlovsk-Kamchatskii, 683600, Russia.

^a Approx average.

remains to be seen whether these will be significant. Even where underexploited resources exist, there may be logistical (e.g. Russia) or roe quality problems (e.g. Australia), which will preclude any significant impact on the world market, at least in the short term.

In the face of wild capture fishery declines around the world, aquaculture has been touted as the panacea. Hagen (1996) reported on the increase in interest in sea urchin aquaculture to meet continued demand and create new commercial opportunities. A range of research activities have been taken in the areas of larviculture, culture system development, as well as roe enhancement and feed development (Parsons, 1997, Lawrence et al. 1997, Grosjean et al. 1998, McBride et al. 1998, Spirlet et al. 1998). Sea urchin aquaculture is, however, in its infancy and remains fertile ground for research to overcome a range of bottlenecks and obstacles to cost-effective commercial production of significant volumes. The continued decline of wild capture sea urchin fisheries is likely to lead to a shortage of supply (particularly of high-quality product) and demand creating significant price incentives to fuel expansion in sea urchin aquaculture. In the absence of complete certainty about

the number of sea urchin fisheries that will be sustainable in the long term and new ones that may develop, it seems prudent for sea urchin aquaculture to address roe enhancement as both cost-effective aquaculture and a tool to aid wild fishery sustainability through value adding of declining outputs through increasing quality.

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**PROCEEDINGS OF
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SHELLFISH SAFETY**

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MANAGEMENT OF SHELLFISH SAFETY IN CHINA

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ABSTRACT This paper describes the fishery environment that affects shellfish production in China. It also provides a brief review of regulations governing shellfish safety and sanitary inspection in China in recent years.

KEY WORDS: Shellfish, red tide, safety

SHELLFISH SAFETY AS INFLUENCED BY RED TIDE

With coastal waters becoming increasingly polluted, the numbers of red tides in China has increased significantly. According to statistical records, red tides occurred three times before the 1970s, nine times in the 1970s, and 29 times in the 1980s. Since 1990, red tides have increased rapidly, with 35 events in 1990, 40 events in 1991, and 50 in 1992. Red tides may cause huge economic losses. In 1989, a red tide on the Bohai Sea Coast, resulted in many fish, shellfish, and shrimp deaths. The total loss amounted to 300 million yuan (RMB). In 1986, at Dongshan Country, Fujian Province, many people were affected by paralytic shellfish poisoning (PSP) after eating the *Venerupis philippinarum* in a red tide event. In this event, one person died, and 59 of 136 people hospitalized were seriously affected. *Gymnodinium* sp. was the major causative organism of this PSP event. Among the 30 genus and 100 phytoplankton species in the China sea, 12 species are related to harmful algal blooms (Table 1).

SHELLFISH PRODUCTIVITY IN CHINA

The shellfish fishery is the major product of seawater aquaculture in China. Its economic value is only slightly lower than that of the catch fishery. Recently, scallops and those species listed in Table 2 have increased outputs. The productivity of cultivated shellfish is shown in Tables 2–4.

REGULATORY MEASURES ON SHELLFISH SAFETY

Safe and sanitary inspection of shellfish products has caught much attention by the general public as well as the regulatory authorities in recent years. After the events of "Arca subcrenate poisoning accident of Shanghai" in 1980s and the shellfish poisoning accident in Fujian province in 1986, government departments have strengthened their management over transportation and distribution of shellfish. Consumers have been warned not to eat uncooked shellfish (especially *Arca subcrenate*).

Because of increasing shellfish poisoning worldwide, China has given increased scrutiny to shellfish safety and has implemented a series of regulations to test for shellfish poisons at the cultivated water, cultured shellfish, and marketed shellfish venues to ensure the safety of shellfish consumption. Because shellfish export from China has increased in the past decade, the Chinese government has also strengthened monitoring and administrative programs to fishery environments to reduce marine environment pollution.

Regulatory Legislation

Marine Environment Regulations Pertaining to Shellfish Safety

Fisheries Law of Peoples' Republic (PR) China;
Environment Protection Law of PR China;
Marine Environment Protection Law of PR China;
Marine Waste Pouring Management Regulation of PR China;
Prevent and Treat Land Pollutants Polluting and Damaging Marine Environment Management Regulation of PR China;
Prevent and Treat Seashore Engineering Construction Project Pollutant and Damaging Marine Environment Management Regulation of PR China, among these, article 7 in Environmental Protection Law and Article 5 in Fisheries Law, provide that fishery service and fishing port supervision and administration agencies should be responsible for supervising waste drainage of ships in fishing ports and monitoring sea water areas of fishery ports areas.

Fishery Bureau

The Fishery Bureau in The Ministry of Agriculture has been establishing "Shellfish Safety and Quality Administration Regulation," to be published in 1998. Its text is divided into the five following parts.

Chapter 1. General.

TABLE 1.

Harmful algal species and their affected areas in China

Harmful Algal Species	Harmful Effects	Sea Areas
<i>Alexandrium tamarense</i>	PSP	Yellow Sea, South China Sea
<i>Alexandrium catenella</i>	PSP	Yellow Sea, South China Sea
<i>Dinophysis acuminata</i>	PSP	Yellow Sea, Bohai Sea
<i>Dinophysis acuta</i>	PSP	Bohai Sea
<i>Dinophysis caudata</i>	DSP	Yellow Sea, East China Sea, South China Sea
<i>Gonyaulax polyedra</i>	PSP	Bohai Sea, South China Sea
<i>Gymnodinium breve</i>	NSP	South China Sea
<i>Gymnodinium sanguineum</i>	NSP	South China Sea
<i>Gymnodinium mikimotoi</i>	NSP	South China Sea
<i>Prorocentrum minimum</i>	DSP	Yellow Sea, East China Sea, South China Sea
<i>Nitzschia pungens</i>	ASP	Yellow Sea, Bohai Sea
<i>Chattonella marina</i>	DSP	Yellow Sea

TABLE 2.
Shellfish type and major culture sea area

Shellfish	Bohai Sea	Yellow Sea	East China Sea	South China Sea
<i>Chlamys farreri</i>	+	+		
<i>Argopecten irradians</i>	+	+		
<i>Patinopecten yessoensis</i>	+	+	+	
<i>Mytilus edulis</i>	+	+		
<i>Mytilus viridis</i>				+
<i>Venerupis philippinarum</i>	+	+	+	+
Oyster	+	+	+	+

Note: + is the shellfish major culture sea area.

Chapter 2. Grade classification, environment supervision, and evaluation of shellfish production area.

Chapter 3. Environment, safety, quality control, and administration of shellfish production area.

Chapter 4. Legal responsibility.

Chapter 5. Attachment.

Establishment of this regulation is intended to protect the ecological environment for shellfish growth, to prevent shellfish from being polluted by shellfish poison and microorganisms, etc., to guarantee profits of fishers, to promote shellfish production, to encourage competitiveness in the market, and to develop international trade.

Environment Standards

China has drawn up and published serious standards concerning water quality of aquaculture and product safety. The food sanitary standard of China provides limits on hazardous metals, detailed in Table 5.

Regulatory Agencies and Relative Responsibilities

The Fishery Bureau of the Ministry of Agriculture, People's Republic of China (i.e., Fishery Service Bureau of People's Republic of China) is responsible for implementation of supervision and administration of fishery environment and shellfish safety. The bureau has established three fishery supervision stations at the Yellow Sea, the Bohai Sea, the East China Sea, and the South China Sea. These are responsible for performing routine fishery environment supervision, especially monitoring of aquachemical and biological environments in fishery waters.

The Yellow Sea and the Bohai Sea are the main shellfish production areas in China. With a cultural area of 600,000 mu = 40,000 ha and their output is 1,300,000 tons per year. The "Shell-

fish Environment Monitoring Regulation of The Yellow Sea and Boahi Sea Area of China," which was enforced in 1998, stipulates that routine monitoring should be carried out once each month in cultural areas and once every 2 months in natural capturing areas.

A red tide monitoring program is implemented to monitor red tide organisms once each month, tracing the changes of red tide daily and monitoring the waters where cultivated shellfish are poisoned.

Monitoring Pollution Events

When a pollution event occurs competent fishery authorities should inform environmental monitoring stations of both local government and the Ministry of Agriculture responsible for performing quality checking. Such checking includes monitoring for water quality and the degree of hazard of shellfish poisoning. This monitoring should be continuous until the red tide disappears.

Reports of Monitoring Results

The monitoring results of each local fishery environmental monitoring station should be reported in a timely fashion to the fishery environment monitoring station of the Ministry of Agriculture of each sea area grade by grade. The environmental monitoring station of the sea area should summarize all monitoring results in the concerned sea area and should report to the fishery service administration bureau of each sea area and the fishery bureau of the Ministry of Agriculture annually.

After receiving the monitoring results, fishery administration branches of each level shall compile and deliver "fishery environment quality report" to all units of shellfish culture, capture, and processing. It must be presented to the fishery administrative branches at the upper levels.

The National Center For Quality Supervision and Test of Aquatic Products P.R. China is an agency under the Ministry of Agriculture. It is responsible for inspection of shellfish products. The main inspection projects include the inspection of indicators of shellfish poison (DSP, PSP), microorganism (aerobic plate count, fecal coliforms, coliforms, salmonella sp., staphylococcus aureus, escherichia coli, V. parahaemolyticus, etc.), and hazardous metal elements, agricultural chemicals, residual medicinal, and organic. It is also responsible for establishment of product quality standards. For example, SC/T 3111-1996 "frozen scallop" has been enacted and in force since December 1, 1996.

The Center has been actively involved in introducing and extending hazardous analysis critical point control (HACCP)—a new method of international fishery quality assurance. The HACCP concerns shellfish safety in the United States and the European Economic Communities. These have been applied to scallop processing and with close supervision from the Center, will supervise and selectively test frozen scallops.

TABLE 3.
Productivities of caught and cultivated shellfish from 1993 to 1996 (ton)

Year	Scallop	Mussel	Clam	Oyster	Razor Clam	Total Culture Output	Catch Output
1993	60,982	37,549	33,042	2,027	2,584	145,727	67,354
1994	825,615	415,222	519,476	313,476	252,886	2,522,755	713,316
1995	916,492	415,179	502,007	373,119	306,749	3,099,099	827,979
1996	999,436	364,643	483,710	373,817	339,848	3,144,131	852,848

TABLE 4.

The cultured shellfish at every sea area from 1993 to 1996 (ton)

Shellfish	Year	Yellow Sea, Bohai Sea	East China Sea	South China Sea
Scallop	1993	718,853	9,494	5
	1994	814,997	10,612	6
	1995	903,079	12,423	990
	1996	486,070	13,202	301
Mussel	1993	424,863	62,823	176,994
	1994	318,894	67,633	28,695
	1995	317,790	70,027	607
	1996	247,771	76,107	40,756
Clam	1993	299,367	98,013	31,373
	1994	353,653	117,405	48,460
	1995	257,160	155,858	89,043
	1996	280,383	146,896	56,257
Oyster	1993	57,393	79,368	31,662
	1994	169,069	92,260	57,777
	1995	153,049	120,344	99,726
	1996	97,179	135,586	144,052

PROSPECTS

There are still some deficits in the monitoring of shellfish safety and sanitation in China. For example, small, white mouse bioassay is mainly used in shellfish poison inspection, despite slow reporting rate and briefness. Because of the great difference among different small, white mouse bodies, and because the requirement of body weight is relatively high for this method, even for bodies with the same weight and physiques, the test error is relatively large. Moreover, this method cannot determine the detailed chemical component of poison in shellfish body.

Relatively, high-power liquid chromatography (HPLC) has high sensitivity. Its major constraint is that there is no pure standard available in China. Moreover, operators must be specially trained for sample processing. For these reasons, this method is not common in China. It is necessary to establish training centers to

TABLE 5.

The chief items in marine water and food standard

Standard Code	Name of Standard	Items	Content
GB11607-89	Water quality standard for fisheries	Coliforms	5,000 cfu/L
		Mercury	0.0005 mg/L
		Cadmium	0.005 mg/L
		Lead	0.05 mg/L
		Chromium	0.1 mg/L
		Copper	0.01 mg/L
		Zinc	0.1 mg/L
		Nickel	0.05 mg/L
		Arsenic	0.05 mg/L
		Cyanide	0.005 mg/L
GB2762-92	Tolerance limit of mercury in foods	Sulphide	0.2 mg/L
		Mercury	0.3 mg/kg
GB4811-84	Tolerance limit in inorganic arsenic in seafoods	Arsenic	2.0 mg/kg
GB14935-94	Tolerance limit of lead in foods	Lead	0.5 mg/kg
GB14961-94	Tolerance limit of chromium in foods	Chromium	2.0 mg/kg
GB15199-94	Tolerance limit of copper in foods	Copper	50 mg/kg

Note: GB is Chinese National Standard.

learn from developed countries. It is necessary to develop brief, rapid, and easily extended shellfish testing techniques suitable for in situ testing in China.

Fortunately, intensive training in various methods of supervision and inspection of shellfish and fishery environments have been begun. Various governmental departments have emphasized that consumers safety, regulations, and systems of sanitation be extensively established. Inspection methods and enforcement teams have also grown. It is hoped that China will be able to provide the best and safest shellfish products to consumers shortly.

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ASSESSMENT OF *PAPHIA UNDULATA* IN NEGROS OCCIDENTAL/GUIMARAS STRAIT WATERS

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ABSTRACT This survey was undertaken from March 1996 to February 1997 to determine the status of the stock and some aspects of the biology of *Paphia undulata* in Hinigaran, Negros Occidental/Guimaras Strait waters. *Paphia undulata* was observed throughout the year, and highest stock density of 29 ind/m² was observed in May, followed thereafter by April, 27 ind/m². Samples of *P. undulata* measured has shell length ranging from 34.00 to 78.00 mm and a mean of 57.65 mm. Length frequency data analysis showed the following growth parameters: L_∞ = 81.50 mm SL, K = (growth constant) 1.2⁻¹. It attains sexual maturity between 40.0 to 50.00 mm SL and has a life span of more than 2 years. Fishing mortality and exploitation rate has a relatively high value, indicating that the stock of *Paphia undulata* in the area is overexploited.

KEY WORDS: Stock assessment, biology, *P. undulata*

INTRODUCTION

Paphia undulata (Born), known in Negros Occidental as “nylon shell” and “badoy” in Sorsogon area, is a commercially important bivalve species in the Philippines. Aside from domestic consumption, its meat is semiprocessed as a chilled and/or frozen and has been exported at a rapidly increasing rate for the last 10 years. To meet increasing demand of the export market, heavy exploitation of the resource by “capandra” fishermen may lead to stock depletion. It was reported by Del Mundo et al. (1992) that, in early 1987, an average of 50 tons were gathered daily in Sorsogon Bay, and gathering ceased when the catch declined in mid-1987 and flourished again in April 1988.

Assessment studies are very important for wise utilization and proper management of this commercially important shellfish to attain sustainable production of *P. undulata*. Baseline information on the relative abundance and biology are vital inputs in developing culture methods to increase production. Today, research works on bivalves in the Philippines are very limited. These include the research of Rusell (1979) on the biology and ecology of *Placma placenta*; Rusell (1984) on the mariculture aspect of kapis; Fortes (1993) on the fishery, biology, and management of economically important shellfish in selected coastal waters of Panay and Negros Islands; and Labe et al. (unpublished report) on the abundance and biology of kapis shell in San Miguel Bay. Available literature on *P. undulata* was only a part of the research undertaken on the commercially important marine bivalves of Sorsogon Bay (Del Mundo et al. 1992).

In Thailand, *P. undulata* had been studied by several fisheries biologists. Bunsopit et al. (1983) and Touycharoon and Benchamarn (1984) reported on the gonadal development and population distributions of *P. undulata*, and Pongthana (1990) on the breeding and rearing of this valuable bivalve. This paper presents the status of the stock and some aspects of the biology of *Paphia undulata* in Hinigaran, Negros Occidental/Guimaras Strait waters.

MATERIALS AND METHODS

Sampling Methodology

Relative Abundance and Production

The sampling area is Hinigaran, Negros Occidental Guimaras Strait waters, bounded by longitude 122°47.5'E and 122°49.5'E

and latitude 10°15.5'N and 10°13.0'N. It has an approximate area of 21 square km, with water depth ranging from 7 to 14 m (Fig. 1). Monthly sampling and landing observations were undertaken. Data on catch composition, total weight and number of catch, actual fishing time, and fishing area were gathered.

Three (3) stations were established in the bivalve shell bed: station I has a water depth of about 9.0 to 10.0 m, station II, 11.0 to 12.0 m; and station III, 13.0 to 14.0 m. A “capandra” fisherman using a compressor was hired to assist the researchers in data gathering. The abundance of *P. undulata* was estimated using quadrat method. An improvised quadrat, 1 × 1 m was laid on the nylon shell beds three (3) times in each station. All shells located within the quadrat were collected. They were counted, and samples were brought to the station for biological studies.

For the land-based data, observations were conducted of one shellfish buyer in the area, and the data recorded were catch per boat and fishing effort. Monthly production was taken from the record of the Fisheries Section, Municipal Agriculture Office, Department of Agriculture (DA), Hinigaran, Negros Occidental.

Biological Data

Shell length was the standard measurement used in length analysis. A total of 4,160 were sampled throughout the survey, their shell length (SL) were measured in millimeters using a Vernier caliper, and grouped in 2 mm length class and used in the analysis of population parameters.

Data Analysis

Estimate of Abundance

The abundance of *P. undulata* is expressed as number of individual per square meter (no./sq. m), and the formula used was:

$$\text{Abundance (no./sq.m)} = \frac{\text{Total number shells}}{\text{Area (sq.m)}}$$

Analysis of Growth Parameters, Rate of Exploitation and Recruitment Pattern

The FAO–ICLARM Stock Assessment Tools (FiSAT) was used in the analysis of length frequency data to estimate growth,



Figure 1. The survey area of *Paphia undulata* in Hinigaran, Negros Occidental, March 1996 to February 1997.

mortality, rate of exploitation, and recruitment pattern. The Powell-Wetherall Plot Method (Powell 1979, Wetherall 1986) was used to estimate the initial value of L_{∞} used in the K-scan routine of the ELEFAN 1 method. The combination of L_{∞} and K-values with the highest goodness-of-fit was used. The expected shell length and growth increments of *P. undulata* were obtained using the ELEFAN 1-output routine. Using the estimates of growth parameters, the total mortality (Z), natural mortality (M), fishing mortality (F), and rate of exploitation were computed using the length-converted catch curve, seasonal recruitment was computed using the recruitment pattern.

RESULTS AND DISCUSSION

Relative Abundance

Paphia undulata was observed throughout the survey period (March 1996 to February 1997), and highest stock density was observed in May 29 ind/m²; followed by April 27 ind/m². The lowest, at 3 ind/m², was recorded in December. An abrupt decrease was noted in June and continued declining until February 1997 (Fig. 2). The average stock density of *P. undulata* in the area is about 5,000 MT.

Decline in catch rates from July 1996 to February 1997 may be attributable to the high fishing effort employed in gathering "nylon shell" in the area. During the first 3 months of the survey, there were about 200 units of fishing boats (with compressors) gathering this valuable marine bivalve with 3 to 4 divers per boat. The number has decreased to about 100 units from June to August, up to about 10 units in February because of minimal catch.

Higher values of catch rate were also observed in May and April, 215 kg/boat and 203 kg/boat, respectively; and January had the least, 51 kg/boat (Fig. 3). Based upon landing data obtained from the Department of Agriculture (DA) Municipal Office, Hinigaran, the highest total production was observed in April at 200.6 MT, followed by March and May, 185.3 MT and 178.4 MT, respectively. The lowest production, about 10.0 MT, was recorded in February 1997. Average monthly production during the peak season (March to May) was about 180.14 MT/month, and 88 MT/month for the rest of the survey period.

Biology

The smallest recorded sample was 34.0 mm shell length (SL), which was recorded in March and May to July; whereas, the large-

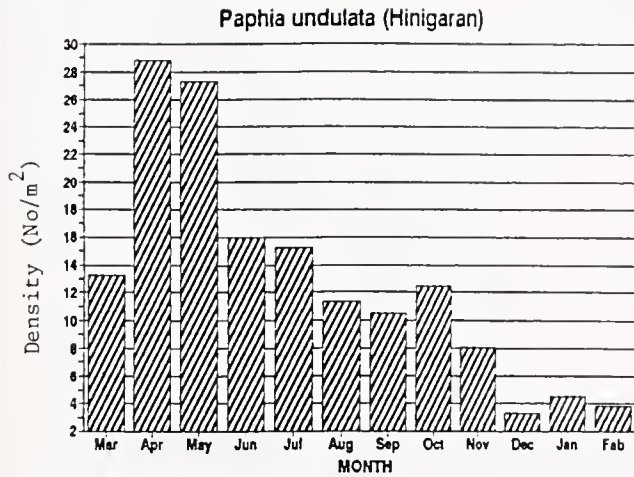


Figure 2. Monthly estimated stock density (n/sq. m) of *P. undulata* stocks in Tagda, Hinigaran, Negros Occidental/Guimaras Strait waters, March 1996 to February 1997.

est sample, with a shell length of 78.0 mm, was noted in August and November. Mean shell length of the pooled samples was 57.65 mm (Fig. 4). In Sorsogon Bay (1990) the minimum size of *P. undulata* measured was 22.0 mm, but the biggest sample was also 78.00 mm SL. Abott (1989) reported that *P. undulata* in Australia has a shell length of 75.00 mm.

As shown in Fig. 5, February samples have the greatest mean shell width (63.50 mm), which was followed by January (61.20 mm); and April, with the smallest (51.30 mm). Shell length analysis of *P. undulata* in the area showed the following population parameters: Loo = 81.50 mm SL, K (growth constant) = 1.2⁻¹ year (Fig. 6). Based upon the growth curve, it seems that *P. undulata* has a life span of more than 2.0 years.

Pongthana (1990) reported that mature *P. undulata* have shell length of 40.0 to 50.0 mm. As shown in Fig. 7, the smallest expected shell length of *P. undulata* was 5.06 mm. Higher growth

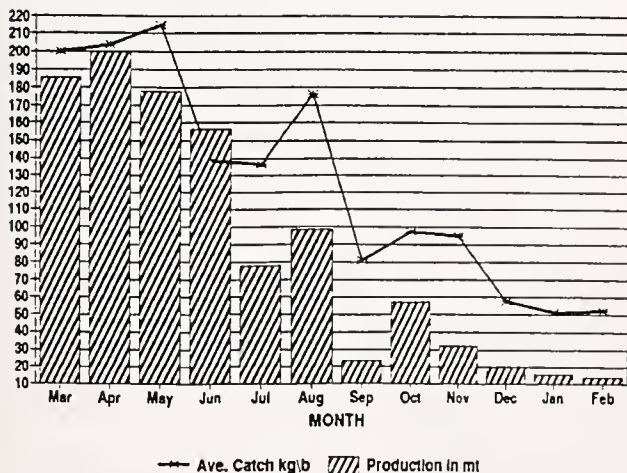


Figure 3. Average catch per boat (kg/boat) based on land-based data, and monthly production of *P. undulata* from the Municipal Agricultural Office (MAO), Hinigaran, Negros Occidental/Guimaras Strait waters, March 1996 to February 1997.

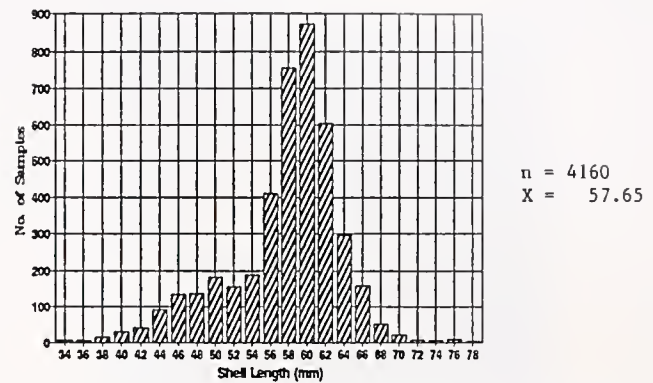


Figure 4. Shell length frequency (pooled samples) of *P. undulata* gathered in Tagda, Hinigaran, Negros Occidental/Guimaras Strait waters, March 1996 to February 1997.

rate was noted before the shells reached their sexual maturity at about 7 months, and has declined as they age. As shown in Figure 8, recruitment patterns suggest that there is only one main pulse of annual recruitment, and this could be supported by the results of the catch rate analysis, where *P. undulata* show only one major peak.

Based upon the results of Pongthana (1990), 1- and 3-month-old *P. undulata* from breeding experiments have shell length of 2.4 and 10.0 mm, respectively. Therefore, it is possible that the smallest expected sample from this survey, 5.06 mm shell length, is about 2 months old, and spawning took place in September. Based upon this result, it seems that the peak of spawning of *P. undulata* in Hinigaran, Negros Occidental, is almost similar to stocks in other countries. In Klong Dan, Samut Prakarn Province (Thailand), peak season of natural spawning of *P. undulata* was observed in August and November (Pongthana 1990).

Mortality and Exploitation Rate

As shown in Figure 9, fishing mortality and exploitation rates are relatively high, indicating a high level of exploitation, and this may be attributable to maximum exploitation of the resource.

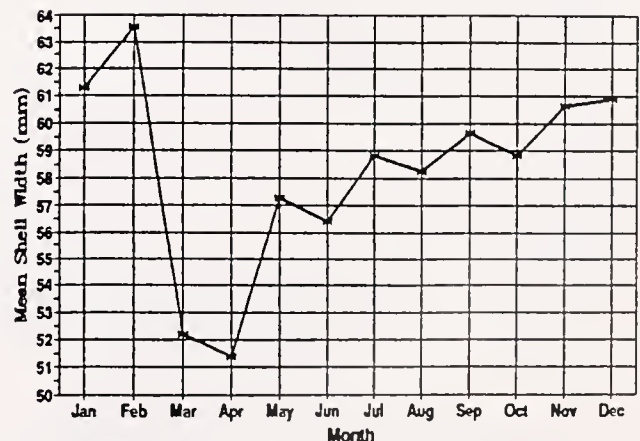


Figure 5. Monthly mean shell length of *P. undulata* samples from March 1996 to February 1997 (land-based and actual samples).

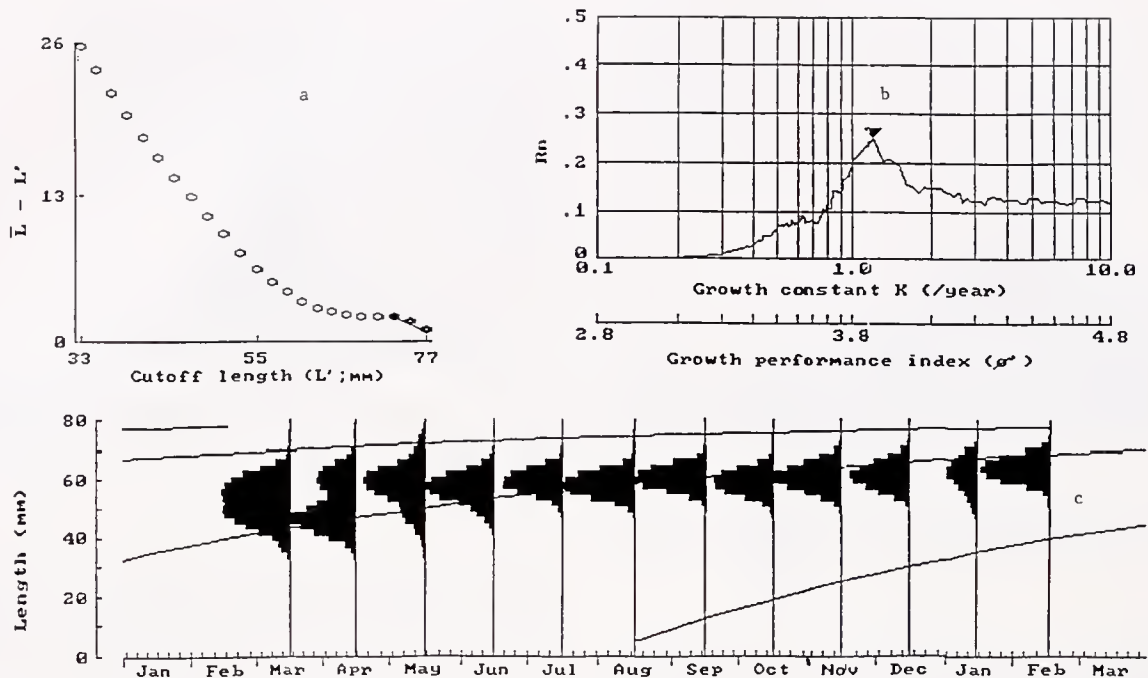


Figure 6. The Powell-Wetherall Plot, $L_{\infty} = 81.00$ mm (a), the result of the K-scan routine, $L_{\infty} = 81.50$ mm, $K = 1.2$ year (b), and growth curve of *P. undulata* samples (c) from March 1996 to February 1997.

Natural mortality in the area can be attributed to habitat degradation caused by:

- (1) Siltation from river runoff during heavy rainfalls—dead shells were gathered at depth ranging from 6.0 to 7.0 m and near the mouth of Hinigaran River.
- (2) Deteriorating water quality—shells gathered in the shallow portion, at about 7 m have a foul odor, and half of the individual shell (posterior portion) has a dark coloration losing its natural color.
- (3) Operation of minitrawl—aside from its very fine mesh net, the operation of minitrawl is considered destructive, because it may cause stress to the organisms thriving in the sea bottom.

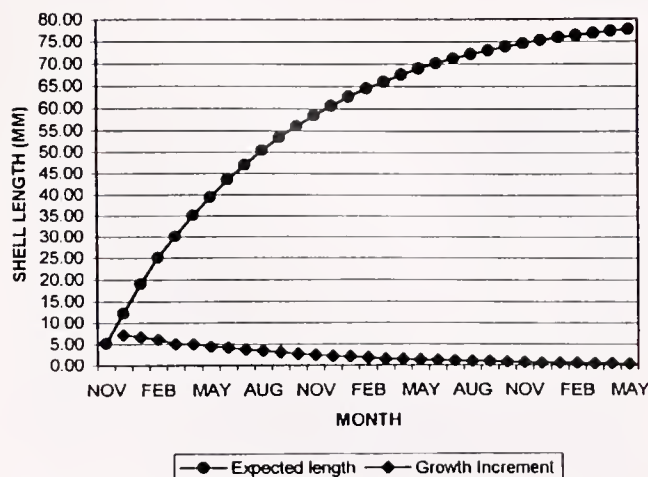


Figure 7. Expected shell length and growth increment of *Paphia undulata* gathered from Hinigaran, Negros Occidental/Guimaras Strait Waters.

Relatively high value of fishing mortality (F) and maximum exploitation ratio could be supported by the continuous decline in catch rates.

CONCLUSION/RECOMMENDATION

Paphia undulata in Hinigaran, Negros Occidental/Guimaras Strait waters has been overexploited because of high fishing effort, leading to recruitment overfishing. At the end of this study, (February 1997) catch rate of *P. undulata* declined to a point no longer profitable for the shellfish gatherer. To have sustainable production of *P. undulata*, the following conservation measures are recommended.

- (1) Fishing effort must be reduced. If there are "too few old fish," the stock is overfished, and fishing pressure on the stock should be reduced (Sparre and Venema 1992). Although some fishery biologists believe it is difficult to implement this management measure, managers should act now to attain sustainability.

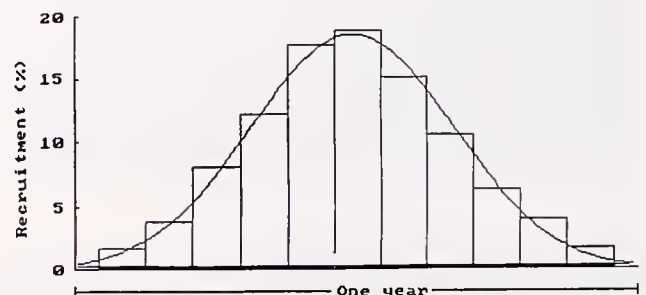


Figure 8. The recruitment pattern of *P. undulata* in Hinigaran, Negros Occidental/Guimaras Strait Waters.

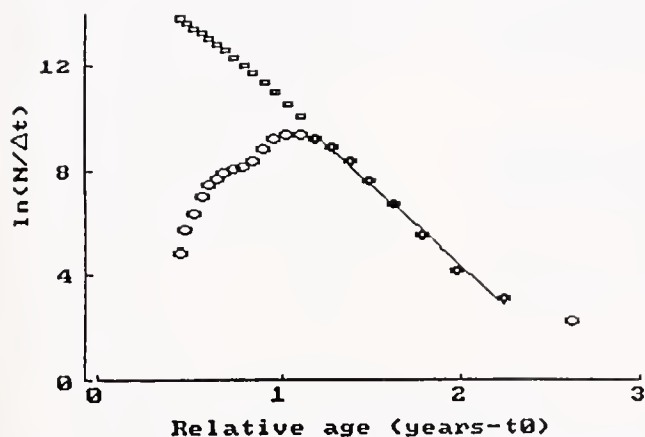


Figure 9. The catch curve of *P. undulata*, total mortality (Z) = 6.22, natural mortality (M) = 2.89, fishing mortality (F) = 3.33, and exploitation rate (E) = 0.54.

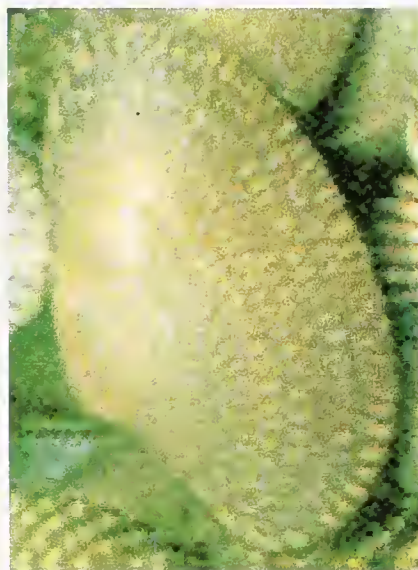


Figure 10. Close-up view of *Paphia undulata* (Born 1778) gathered from Hinigaran, Negros Occidental. Taxonomic classification: Class: Bivalvia; Order: Veneroida; Superfamily: Veneracea; Family: Veneridae; Genus: *Paphia*; Species: *undulata*; Scientific Name: *Paphia undulata* (Born 1778).

- (2) Establishment of fish sanctuary.
- (3) Strict implementation of fishery rules and regulations, such as the operation of minitrawl using fine mesh net and commercial trawlers in municipal waters
- (4) The local government should coordinate with the Department of Environment and Natural Resources (DENR) to determine the extent of water pollution, and antipollution laws should be strictly implemented. Forest regulations should also be strictly enforced to prevent soil erosion that may cause siltation.
- (5) To meet the increasing demand of *P. undulata* and to prevent overexploitation of the wild stock, the Aquaculture Division of this bureau, and the Fisheries Section, Department of Agriculture, Negros Occidental should propose a project on breeding and aquaculture.

ACKNOWLEDGMENTS

The authors thank our Division Chief, Mr. Jose A. Ordonez, our Director, Dennis B. Araullo, and the Hon. Mayor, Ricardo Presbitero of Valladolid, Negros Occidental, for their invaluable support. We also extend our gratitude to the staff of the Department of Agriculture, Hinigaran, especially to Mr. Dimple Guanzon for providing us the monthly production data and to our colleagues Leony L. Mijares and Rosarie G. Arreza for their assistance in data gathering.

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SHELLFISH TOXICITY AND *PYRODINIUM* CELL DENSITY IN BATAAN, PHILIPPINES (1994–1997)

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ABSTRACT Cell density of *Pyrodinium bahamense* var. *compressum*, a paralytic shellfish poisoning (PSP) causative organism and toxicity of *Perna viridis* off Bataan (Manila Bay, Philippines) during 1994 to 1997 were monitored based mainly on the samplings conducted by the Bataan Red Tide Testing Center. Maximum *Pyrodinium* cell counts were observed during the months of June to August (1994–1996) and minimum during the colder months of November to February (1994–1996). The toxicity of green mussels, *Perna viridis*, as determined by mouse bioassay was highest during the months of June and July (1994–1996) and lowest during November to February of the same years. From January to October 1997, there was no *Pyrodinium* bloom and, consequently, no shellfish toxicity.

INTRODUCTION

Pyrodinium bahamense var. *compressum* has been the major causative organism of toxic red tides in the Philippines and some other countries in the Indo-Pacific areas (Corrales and Maclean 1995). Aside from health risks caused by consumption of contaminated shellfish, economic dislocation primarily from loss of livelihood has been experienced by people, especially those in shellfish industry. Moreover, the "red tide scare" could cause further losses of income to fishermen, because fish, crustaceans, and other mollusks could not be marketed during red tide seasons.

In Bataan, shellfish, particularly green mussels, are both grown and harvested from the wild. Shellfish growers from the east side of Manila Bay, such as Navotas and Parañaque, usually get their spats or juvenile shellfish from Bataan. A Bataan Red Tide Testing Center (BRTTC) was established in 1993 in line with the national government's effort to conduct monitoring activities at affected sites. Considering the limited resources and available trained manpower, the BRTTC was established by the Department of Science and Technology (DOST) on the campus of Bataan School of Fisheries (BSF) in Orion, Bataan.

The establishment of the BRTTC became imperative with the yearly occurrence of toxic red tides in several coastal areas in Manila Bay, particularly in Bataan. The time lag from generating data through the monitoring activities of the Center to giving advice to the consuming public has been shortened, thus, information dissemination has been facilitated.

MATERIALS AND METHODS

Regular sampling was done in the BRTTC sampling stations; namely, Capunitan and Puting Buhangin in Orion; Bo, Luz, Bataan Refining Corporation (BRC) and Planters in Limay, Bataan (Fig. 1).

Plankton samples were taken by vertical net towing, and green mussels were collected in the five stations from January 1994 to October 1997. The samples were then brought to the Center for analyses.

Green mussels were processed and analyzed for paralytic shell-

fish poisoning (PSP) toxin level using the standard mouse bioassay procedure (AOAC 1984). *Pyrodinium* cell density was estimated using a Sedgwick Rafter Counter with the modified formula of Guillard (1972).

RESULTS AND DISCUSSION

Statistical analyses (ANOVA with three sources of variation) show that cell density of *Pyrodinium bahamense* var. *compressum* and monthly averages of *Perna viridis* toxicity did not vary significantly with sampling stations.

Using Duncan's Multiple Range Test, the 1995 *Pyrodinium* bloom was found to be significantly higher than 1994 and 1996 blooms. The major peaks of the bloom occurred in the months of June and July. Highest *Pyrodinium* cell counts were found during the months of June to August, and the lowest concentrations were found during the months of November to February. Since 1994, *P. bahamense* populations have been observed annually, except for 1997, in maximum concentrations between June to July; for example, 10^5 cells/L was observed on June 1995 in Capunitan station (Table 1).

In the Philippines, for the period 1991 to 1995, blooms have occurred in June to July (early southwest monsoon), which coincided with periods of maximum rainfall that increase thermal stratification and vertical stability of the water column; whereas, cyst densities in the sediment were highest during the northeast monsoon (Villanoy et al. 1995).

P. viridis toxicity was highest in 1994 and lowest in 1995. Its toxicity was significantly higher in June and July than in all other months. Shellfish toxicity usually occurred during the months of June to September. Since 1994, the highest toxicity observed from green mussels *Perna viridis* was 6,877 $\mu\text{g}/100\text{ g}$ shellfish meat in June 1994 from Puting Buhangin station.

Annual maximum shellfish toxicity generally occurred concurrent with the *Pyrodinium* bloom episodes, corroborating the observations of Bajarias and Relox (1996). This is to be expected, because *Pyrodinium* was the major causative organism/source of paralytic shellfish toxins in Bataan waters during the period.

According to Cembella et al. (1988), a high number of toxi-

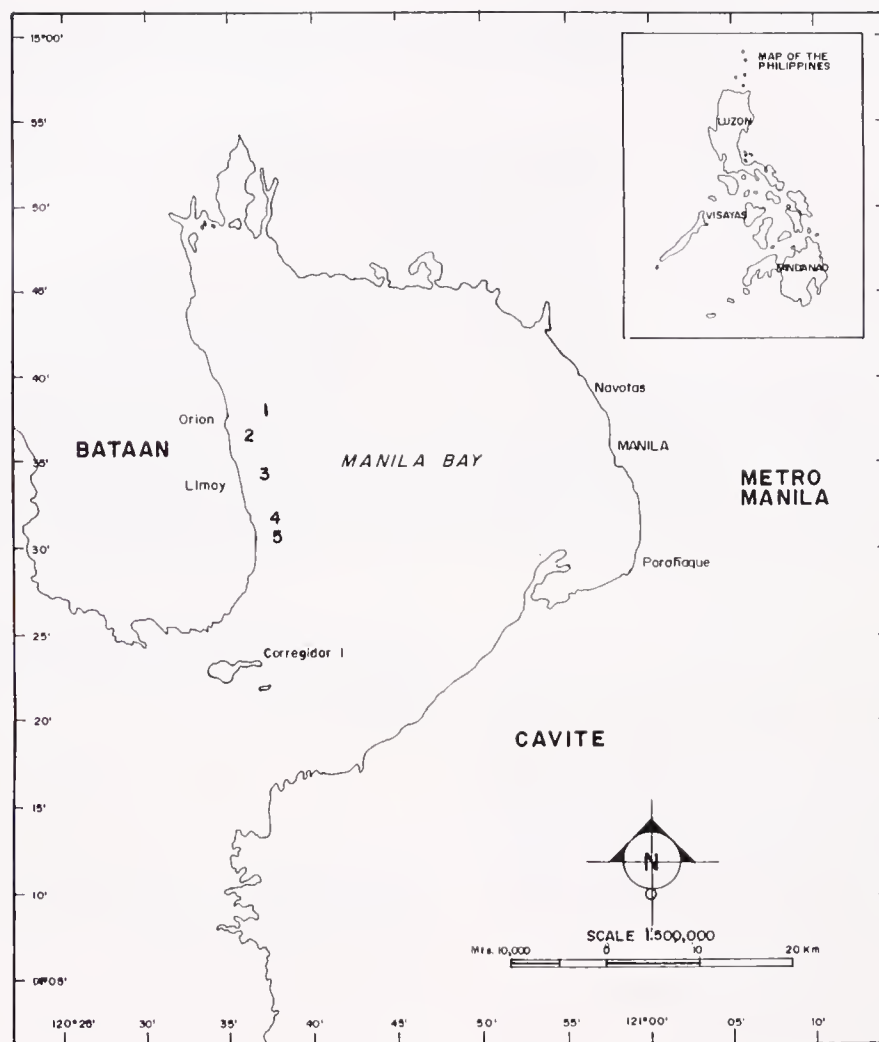


Figure 1. Map showing the study sites in Bataan area, Manila Bay, Philippines (1, Capunitan; 2, Puting Buhangin; 3, Bo. Luz; 4, BRC, and 5, Planters).

TABLE 1.

P. bahamense var. *compressum* abundance and *Perna viridis* toxicity in Bataan waters from 1994 to 1996.

Site	Year	Maximum <i>P. bahamense</i> Conc. (Cells/L)	Maximum PSP Conc. (µg/100 g)
Capunitan	July 1994	No data	3,207
	June 1995	10^5	1,880
	July 1996	690	2,216
P. Buhangin	June 1994	Incomplete data	6,877
	July 1995	10^5	1,953
	June 1996	1,250	1,981
Bo. Luz	June 1994	129	3,912
	July 1995	10^5	1,039
	June 1996	150	750
BRC	June 1994	144	5,432
	July 1995	10^5	1,319
	July 1996	3,570	1,242
Planters	June 1994	152	4,504
	June 1995	10^5	1,990
	July 1996	2,100	2,106

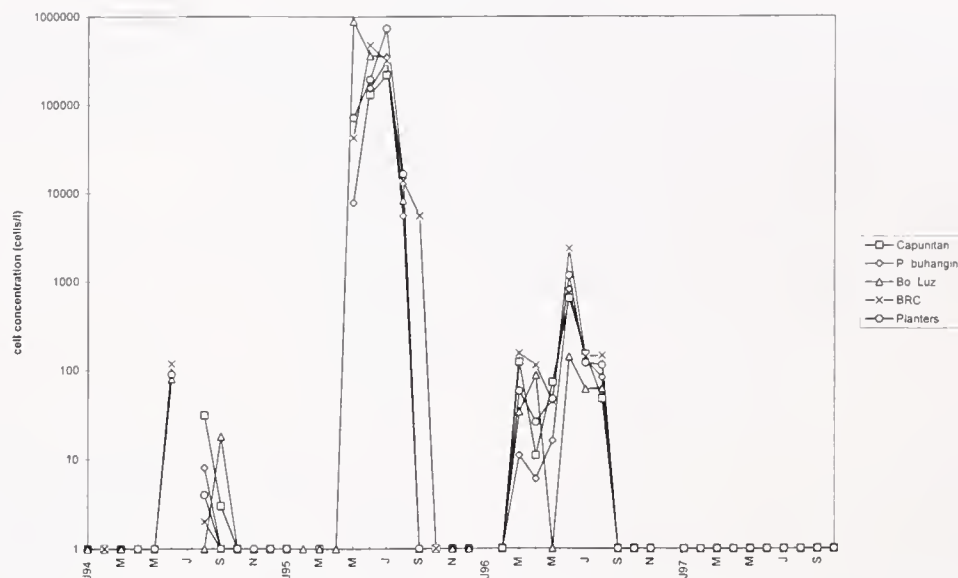


Figure 2. Monthly averages of *Pyrodinium bahamense* var. *compressum* cell density in five sampling stations in Bataan area, Manila Bay, Philippines 1994 to 1997.

genic cells cannot be used to predict mollusk toxicity in certain areas. Bricelj and Shumway (1997) discussed some of the factors influencing toxin accumulation by bivalves. Bloom characteristics (cell density, specific toxicity, and bloom duration), prior history of exposure to PSP toxins where some bivalve populations repeatedly exposed to PSP toxins might become more resistant and accumulate higher toxin levels than those with no prior contamination, and such sources of intrapopulation variability as variation in sensitivity to PSP toxins among individuals from the same sampling stations, variation in body mass of bivalves, and imprecision in the mouse bioassay (ca. $\pm 20\%$, Adams and Furfari 1984).

There have been no recorded blooms of the organism and shellfish toxicity in 1997. As more research is carried out, it may

become evident that meteorological events (such as the El Niño phenomenon) could modify the physicochemical properties of the sediment and water column, leading to conditions unfavorable for *P. bahamense* growth and bloom (Usup and Azanza 1997).

For example, throughout the years 1992 to 1993, sea surface temperatures of waters in New Zealand continued a downward trend because of the effect of an El Niño phase of ENSO (Ballantine 1992). Widespread shellfish poisoning and algal blooms coincided with this unusual weather. The two most widespread and most abundant toxic dinoflagellate species, *Gymnodinium* sp. and *A. minutum*, occurred in early 1993 in New Zealand after a prolonged period of westerly and southwesterly winds, an effect of the El Niño event (Chang et al. 1995).

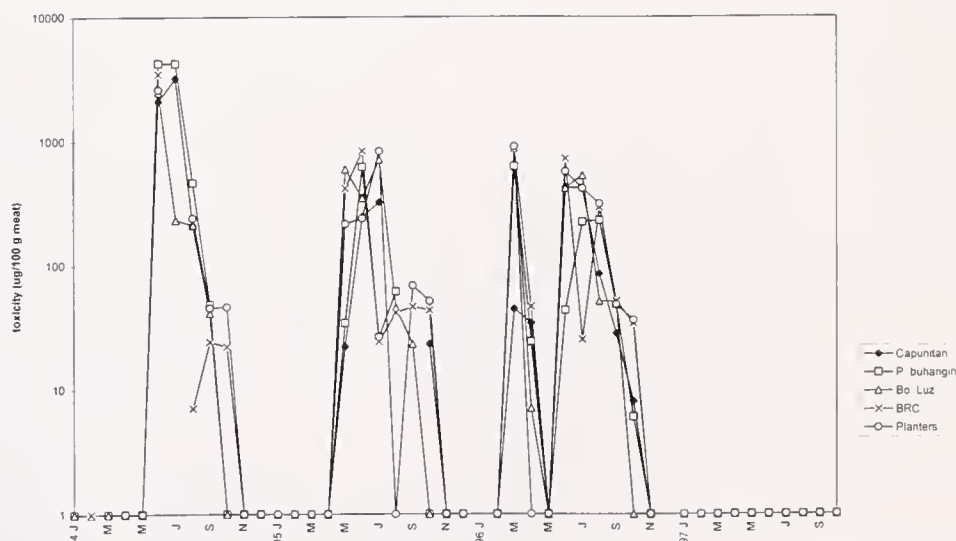


Figure 3. Monthly averages of *Perna viridis* toxicity in five sampling stations in Bataan area, Manila Bay, Philippines 1994 to 1997.

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INITIATING AND TRIGGERING MECHANISMS CAUSING HARMFUL ALGAL BLOOMS

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ABSTRACT Algal blooms have been variously associated with such environmental factors as excessive eutrophication, but the actual mechanisms that eventually lead to excystment have never been completely understood. Using weather data and observations of bloom events involving *Pyrodinium bahamense* var. *compressum* in Manila Bay as a case study, the role of waves in the formation of the “seed bed,” the suspension of cysts, and the transformation of cysts into vegetative cells is presented and discussed. Results suggest that waves generated by onshore wind may be important in the onset of *P. bahamense* blooms. These findings are significant, because they not only advance current knowledge about the life cycle of *P. bahamense* but also provide the possible missing links that have long been hindering the prediction of algal events involving other cyst-forming dinoflagellates. The results of the study may also be useful when formulating alternative strategies toward: (1) managing harmful algal blooms in existing mussel and shellfish culture areas; and (2) selecting new culture sites to ensure mussel and shellfish safety throughout the world.

KEY WORDS: “seed bed” formation, harmful algal blooms, *Pyrodinium bahamense* var. *compressum*, prediction, shellfish safety

INTRODUCTION

The Philippine shellfish industry is persistently affected by the nearly annual incidence of harmful algal blooms (HABs) since 1983. As of 1990, the production of mussels and oysters reached 17,515 MT and 13,485 MT, respectively, collectively contributing about 5% of total aquaculture production (BAS 1990). At current prices, this would be about PhP 320 M, or close to US \$10 M. *Pyrodinium bahamense* var. *compressum* is the main causative organism for HABs throughout the country. However, reports from Manila Bay during the past 2 years now indicate the presence of the temperate toxic dinoflagellate *Gymnodinium catenatum*. Cysts of other dinoflagellate species, including *Alexandrium* sp. and *Gonyaulax* spp., have also been occasionally noted in the area (Matsuoka pers. comm.). Until 1996, 1,768 paralytic shellfish poisoning cases, resulting in 107 deaths, had been reported in about 20 different bodies of water across the country (BFAR, unpublished data) and severely affected the livelihood of fishers.

This study is part of an independent and continuing investigation toward formulating alternative protocols for managing and mitigating HAB events in Philippine waters to ensure shellfish safety. This portion of the investigation aims to identify the mechanisms that cause algal blooms. It seeks to address the hypothesis that waves are mainly responsible for both “seed bed” formation and cyst suspension. Understanding these mechanisms will lead to a shift from “reactive” management and mitigation protocols to “predictive” programs. These results are also expected to help address the harmful impacts of future algal episodes on the shellfish industry.

MATERIALS AND METHODS

The proposed hypotheses were tested using data from Manila Bay, which is located in Luzon, Philippines (Fig. 1). This site was chosen, because documentation of past HAB episodes had already

been made (Bajarias and Relox 1996, Corrales and Crisostomo 1996, Villanoy et al. 1996). Rainfall (Fig. 2) and wind velocity (speed and direction) (Fig. 3) recorded over a 3-year period from the PAG-ASA station in Port Area, Manila were examined. Links between these weather observations and previously documented algal bloom events were made through known relationships between wind and waves (Kinsman 1965) and the subsequent effects of waves on cysts.

RESULTS AND DISCUSSION

The weather recorded by PAG-ASA in Manila Bay exhibited consistent patterns during the 3-year study period from January 1991 to December 1993. Rainfall was practically absent during the first half of each year but was usually highest during the third quarter (Fig. 2). Rainy days, however, sometimes extended toward the end of the year. A frequency histogram of wind speed, meanwhile, revealed that winds of 2 to 3 m/s were dominant (Fig. 4). Furthermore, the contour plot of directional wind frequency for the entire 3-year period (Fig. 5) yields the following results: (1) the dominant wind direction during the first quarter normally shifted from northeasterly winds (292.5°) to southeasterly winds (247.5°); (2) from May to September, which corresponds to the rainy season, southwesterly winds (157.5°) were dominant; and (3) the transition of prevailing wind directions varies in a clockwise manner.

Incidentally, in a study of Corrales and Crisostomo (1996) that covered a period of 14 months from April 1993 to May 1994, it was observed that cyst density was highest from April to July 1993 along the western coast of Manila Bay. This is apparently caused by the effects of waves generated by winds that were persistently blowing between 247.5 to 292.5° from February to June in 1993. To explain this relationship more clearly, note that winds blowing at speeds as low as 1 m/s can generate waves with different wavelengths (Bretchneider 1965, citing Jeffreys 1925). Waves influence



Figure 1. Map showing the location of the study area.

the movement of bottom particles if the water depth is less than half their wavelengths (CERC 1984). In particular, onshore wind-generated waves moving toward shallow waters are responsible for the mass transport of sediments, together with cysts that may be present, in their propagation direction (CERC 1984). Undoubtedly, this mechanism, which had long been observed to affect sediment dynamics in coastal waters (see e.g., Horikawa 1978), is also responsible for the accumulation of cysts into "seed beds" (Steidinger 1975) from which algal blooms of cyst-forming dinoflagellates such as *P. bahamense* may start to germinate in shallow waters.

As with "seed bed" formation, the effect of wind-generated waves on cysts before suspension should not significantly differ from that of silt because of their similar specific gravities. To see this, note that in shallow water, waves propagating toward the coast become steeper and eventually break. Those with longer wavelengths break farther offshore. The impact of breaking waves near the coast suspends sediments, particularly small and light

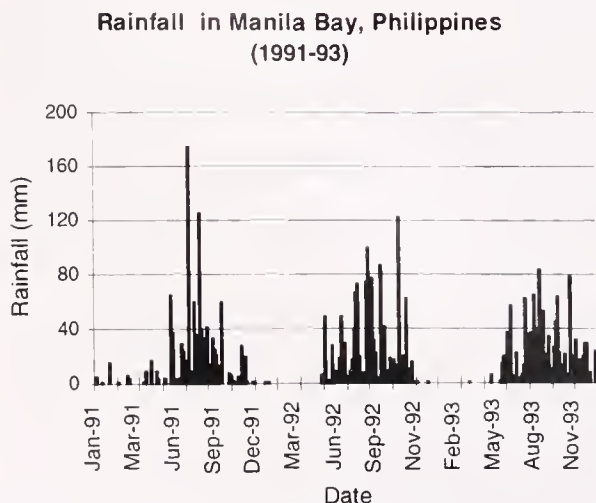


Figure 2. Daily rainfall recorded at Port Area PAG-ASA Station in Manila Bay, Philippines from January 1, 1991 to December 31, 1993.

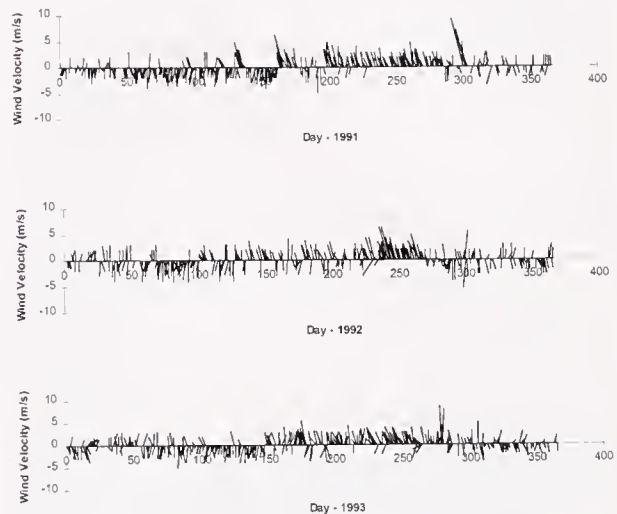


Figure 3. Daily wind velocity (speed and direction) recorded at Port Area PAG-ASA Station in Manila Bay, Philippines from January 1, 1991 to December 31, 1993.

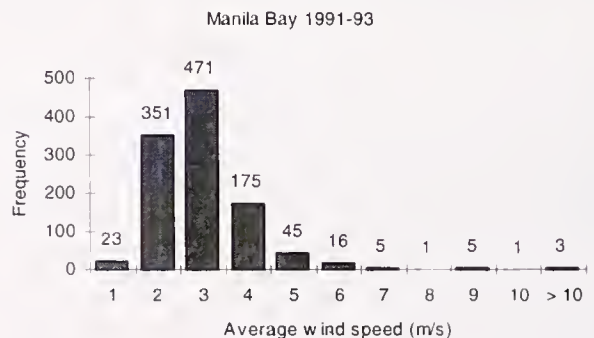


Figure 4. Histogram of average daily wind speed at the Port Area PAG-ASA Station, Manila, Philippines from January 1, 1991 to December 31, 1993.

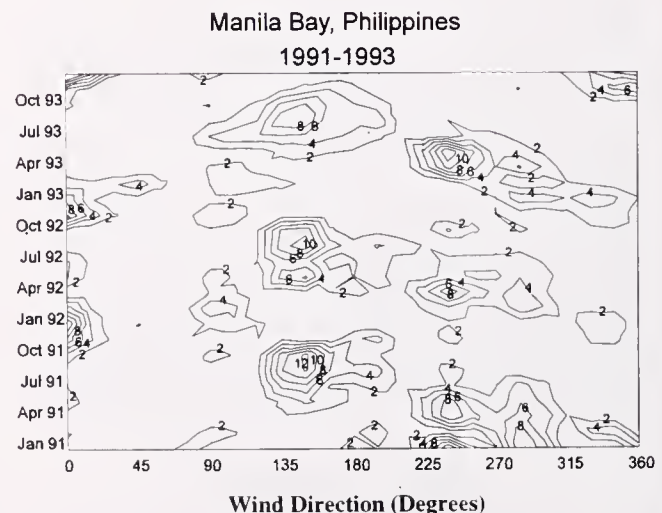


Figure 5. Contour plot of directional wind frequency recorded in Port Area PAG-ASA Station in Manila Bay, Philippines from January 1, 1991 to December 31, 1993.

Year	J	F	M	A	M	J	J	A	S	O	N	D	SOURCE
Wind													this study
1991													
Motile cell													Bajarias & Relox 1996
Wind													this study
1992													
Motile cell													Bajarias & Relox 1996
Wind													this study
1993													
Cysts													Corrales & Crisostomo 1996
Motile cell													Bajarias & Relox 1996

Figure 6. *Pyrodinium* bloom period relative to predominant wind direction in Manila Bay, Philippines from 1991 to 1993.

particles such as mud and silt. Therefore, waves breaking near "seed bed" sites would also be expected to suspend cysts. It should be pointed out that although turbulent mixing had been postulated as a possible suspending mechanism for cysts (Villanoy et al. 1996), the impact of more intense processes, such as breaking waves, offer a more plausible explanation. It is also significant to note that waves are an important source of turbulent energy.

It is apparent from this study that wave-induced resuspension of cysts from the bottom may be an important precursor in the formation of blooms. This can be seen by comparing the foregoing conclusions with the results of a government red tide monitoring team. From January 1992 to December 1994, the average concentration of phosphate across Manila Bay was fairly consistent at about $1.0 \mu\text{g-atom L}^{-1}$; whereas, vegetative cells of *P. bahamense* were initially detected off Bataan during the first quarter of each year (Bajarias and Relox 1996). During this period, rainfall in Manila Bay was practically nil (see Fig. 2). The detection of *P. bahamense* vegetative cells in March 1992 and 1993 (Bajarias and Relox 1996) apparently follows the onset of persistent onshore wind from January to April (see Fig. 5). Note that there is usually a time lag between the observed onset of blooms and the period during which wind blows persistently toward the shore (Fig. 6). This time lag probably represents the period that is required for the accumulation of cysts to form "seed beds," and their subsequent resuspension from the bottom by onshore wind-generated waves, prior to excystment. Additional observations are clearly needed to establish the actual time lag between both events precisely.

The foregoing findings are significant, because the possible role of waves in triggering *Pyrodinium* blooms has been inadequately explored and has not been previously proposed (see e.g., Paerl 1988). It also contradicts a previous explanation of Gonzales (1989) that upwelling processes are responsible for *Pyrodinium* blooms in Manila Bay. Possibly, *Pyrodinium* blooms are initiated by the increased sensitivity of their cyst's receptors to such triggering factors as light once they get suspended in the water column. Other information that can be derived from the weather data in relation to observed algal events in Manila Bay is the fact that the changing wind direction following a clockwise pattern suggests the transport of newly germinated vegetative cells from Bataan to Cavite, where the major shellfish culture sites exist. These results are expected to help address the harmful impacts of future algal episodes on the shellfish industry in the bay.

Results of the study suggest that more factors may actually be involved in *Pyrodinium* blooms. It is clear that these seemingly

natural phenomena can happen even during the dry season. There is reason to believe that excessive erosion in upland areas, which usually leads to heavy sedimentation in the coastal zone, probably needs closer consideration. To see this, it must be noted that excessive sedimentation could wipe out such natural marine habitats as corals and seagrasses, thus forming a bare sea bottom. Under this condition, sediments and cysts that are freely acted upon by wave action could also promote algal outbreaks. As proof, note that past HABs usually occurred where corals and seagrasses are either degraded by sedimentation or absent, as in Maqueda Bay in eastern Philippines. Where both seagrasses and corals thrive, as in Guimaras Island (Babaran et al. 1997a, Ingles et al. 1997), no algal blooms have been reported, despite the noted presence of *Pyrodinium* cysts (Babaran et al. 1997b). Therefore, attempts to address HABs should incorporate the issue of environmental degradation in the watershed.

CONCLUSION

This study provides new and controversial insights about the role of waves in "seed bed" formation, cyst suspension, and triggering of algal blooms. It yields a clearer view of the life cycle of cyst-forming dinoflagellates and presents an alternative explanation to one of the most confusing stages in the life cycle of *P. bahamense* var. *compressum*. Specifically, the study suggests that onshore, wind-generated waves have a significant role in the formation of "seed beds" and the resuspension of resting cysts from the sea bottom, which initiate the onset of algal blooms. This conclusion, however, requires further investigation. Furthermore, algal blooms seem to recur, because degraded environmental conditions apparently favor the completion of the life cycle of such causative organisms as *P. bahamense* var. *compressum*. Thus, to manage and mitigate the harmful impacts of algal blooms on shellfish, the conservation of such natural marine habitats as corals and seagrasses should not be overlooked. Moreover, long-term solutions should strongly consider the integrated management of terrestrial and marine environments.

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THE USE OF PHYSIOLOGICAL ASSESSMENT TECHNIQUES FOR DETERMINING THE RELATIVE ACTIVITY RATES OF BIVALVE SHELLFISH DURING SIMULATED DEPURATION

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ABSTRACT A series of trials were undertaken over an 18-month period to determine the relative activity rates for mussels (*Mytilus edulis*), Pacific oysters (*Crassostrea gigas*), native oysters (*Ostrea edulis*), cockles (*Cerastoderma edule*), and Manila clams (*Tapes philippinarum*) subjected to varying seawater temperatures and dissolved oxygen levels. All these species are currently commercially depurated in the UK. To achieve this, alternative techniques to the more traditional use of bacteriological analysis were used to establish the physiological response of bivalve mollusks to varying conditions. These were the monitoring of ammonia excretion, consumption of dissolved oxygen, and uptake of a neutral red dye. The monitoring of ammonia excretion correlated with dissolved oxygen consumption, and these proved to be useful methods of obtaining information on the physiological response of bivalve mollusks subject to varying simulated depuration conditions. The information obtained could not have been achieved by bacteriological analysis. However, the dye test, although already an established method, did not prove to be entirely satisfactory. Overall, the results found that both species of oyster were much less active than the other species, which may have implications for depuration systems.

KEY WORDS: depuration, physiology, activity rate, mussels, oysters, cockles, clams

INTRODUCTION

There is a known health risk associated with eating bivalve mollusks, especially those eaten raw or lightly cooked. Epidemiological evidence (Lées 1996, CEFAS 1997) suggests that in the UK and other developed countries, outbreaks are predominantly caused by small, round-structured viruses (SRSVs) of the Norwalk or Norwalk-like family that cause gastroenteritis. Viral, not bacterial, infections are the predominant cause of infectious disease following shellfish consumption. The main species of shellfish implicated in England and Wales during recent years has been oysters.

This health risk is minimized and regulated by UK Food Safety Regulations (SI 1508 and SI 3164), which are derived from EU Directive 91/492/EEC. These regulations require that bivalve mollusks taken from a category B classification area (the area most commonly found in England and Wales) must be treated by either controlled cooking, relaying, or depuration. Depuration is the industry's most commonly preferred option.

The prescribed operating criteria for depuration systems are based upon seawater quality, temperature, and flow and the loading density of the mollusks in the system. Much of the scientific research into these criteria in the UK was undertaken many years ago, at a time when only mussels and native oysters required depuration. Other species have been introduced, and the existing criteria have been applied (as in the case of Pacific oysters) or criteria adopted from another country (as with clam species). To date, much of the scientific investigation of purification criteria has been made on the basis of bacterial reduction of indicator organisms. However, such bacteriological analysis usually gives only a positive or negative result; that is, mollusks either depurated or they did not. It does not necessarily indicate how much better one seawater temperature is than another. Bacteriological analysis is also expensive and has the added uncertainty associated with the need to ensure high initial bacterial counts in the mollusks.

A series of trials were carried out to establish alternative physiological assessment methods for determining the activity rate of

commercially harvested bivalve mollusks in simulated depuration conditions and then to compare the relative activity rates of five species of commercially harvested bivalve mollusks, at varying temperatures. Three methods were used to assess the physiological response of bivalve mollusks. These were the measurement of ammonia excretion, dissolved oxygen (DO) consumption, and the removal of dye from water. Ammonia excretion has been shown to correlate with fecal deposition in mussels (Hawkins et al. 1983), but this method has previously had only limited use in depuration investigations. In the context of this work, ammonia excretion is considered to be more an indicator of general metabolic activity. The consumption of DO represents respiration activity. The uptake of neutral red dye indicates filtration activity (Cole and Hepper 1954).

MATERIALS AND METHODS

A test unit supporting four standardized model depuration tanks was constructed for the trials. The mollusk test unit consisted of four identical test tanks, each with independent seawater circulation, aeration, and temperature control systems, mounted on a bench, as shown in Figure 1. Each tank consisted of a plastic Allibert type 12030 stack nest box of 30 L capacity and effective internal working length of 650 mm, width 370 mm, and depth of 110 mm. Seawater was drawn from one end of the tank to the other via a magnetically coupled pump. No spray bar was used, the water re-entering the tank below the water surface to prevent any re-oxygenation. Instead, aeration was supplied by a solenoid air pump feeding a porous tube aerator laid across the bottom of the tank at the water input end. Air supply was then controlled by an Oxyguard DO monitor, with a probe in the center of each tank that controlled the air flow to meet the required level of DO. Seawater could be heated by the use of thermostatically controlled titanium rod type heaters. A type 316 stainless steel immersion coil fitted to each tank provided chilling via a beer chilling unit, with a separate supply of water circulating through the chilling system.

Each tank was filled with 24 L of seawater at the required

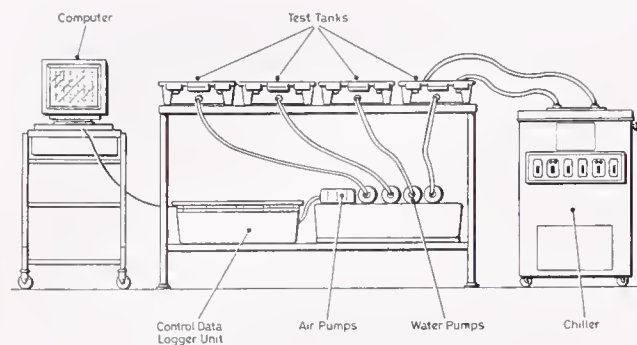


Figure 1. Mollusk test unit.

temperature and 5 kg (in-shell weight) of mollusks. An exception to this was made for cockles, which, because of their potentially high level of activity (Boulter et al. 1994), were limited to 2.5 kg. Each tank operated at a water flow rate of 10 L/min. Following trials with each batch of mollusks, some were shucked to obtain meat yield. After each trial, the tank and its associated pipework was thoroughly cleaned and flushed.

The accumulation of ammonia in the water was monitored using Dr. Lange type LCK 303 and LCK 317 test kits for ammonium nitrogen (NH_4N). Absorbency was measured on a HACH DR2000 spectrophotometer at a wavelength of 695 nm. The Dr. Lange kits were calibrated against ammonium nitrogen artificial seawater standards.

For each trial, the seawater was tested before placing the mollusks in the tank. The mollusks were then immersed in the seawater. Temperature was allowed to settle at the required levels, and DO was maintained at above the 80% saturation level. The seawater was tested after 24 hours and at the end of a simulated depuration period of 42 to 48 hours.

The approach to measurement of DO consumption was to load the tanks with mollusks and then run them for an extended period (at least overnight and normally 2 days) to stabilize the conditions and then to switch off the air supply. The single DO probe in each tank was then used to monitor the reduction in DO over a period of 1 hour. This was often used on completion of an ammonia monitoring trial. It was done at different seawater temperatures to obtain a relative measure of the variation in activity against temperature. DO was measured using a multiprobe Oxyguard DO meter connected to a data logger measuring in ppm.

The method used for dye removal was based upon monitoring the rate at which neutral red dye is removed from the water. The mollusks filter the dye out of the water and into their gills, which stain red. 30 mL of a 1% solution of neutral red dye was mixed with 1 L of seawater taken from the mollusks tank. This solution was then mixed with the rest of the water in the tank. The water in the tank was then sampled at half-hour intervals for the first 2 to 3 hours and then at hourly intervals up to 6 to 7 hours. Its absorbency was assessed at 495 nm, the test level for assessing turbidity in FTUs (formazin turbidity units), using a HACH DR/2000 spectrophotometer. These trials were carried out on a similar basis to the oxygen consumption trials with the tanks running for an extended period to stabilize conditions before the dye was added.

Artificial seawater (ASW) made up to a seawater salinity of 30 parts per thousand (‰) was used throughout the trials. A standard premix containing the five main constituent salts of seawater was used, obtained from Peacock Salts, Glasgow, Scotland. A fresh mix of ASW was used for every trial.

Five species of bivalve mollusk were obtained from commercial suppliers in the UK. These were: cockles (*Cerastoderma edule*), Manila clams (*Tapes philippinarum*), mussels (*Mytilus edulis*), native oysters (*Ostrea edulis*), and Pacific oysters (*Crassostrea gigas*). Care was taken to ensure that all mollusks used were hand gathered, freshly harvested, and not subject to any unnecessary physical shock or delay in transport. On receipt at the laboratory, any gaping or damaged mollusks were discarded, and any barnacle encrustation was removed. Sufficient shellfish were usually obtained to carry out two or three consecutive trials sequences within 1 week. They were held immersed until required in a 600-L Seafish designed small-scale depuration tank, which was used as a storage tank, at a temperature of 12°C. Mollusks were always conditioned in this holding tank for a minimum of one night before trials to allow them to acclimatize to tank conditions and to allow them to excrete any ammonia accumulated in their intravalvular fluid as a result of dry storage during transportation to the laboratory. Mollusks for each trial were taken from the tank, debysed, if necessary, sorted to remove any further dead or damaged specimens, washed, weighed, and then placed directly in the test unit. After each trial, the particular mollusks used in that trial were disposed of, and fresh mollusks were used in the subsequent trial. The trials were carried out over a period of 13 months between November 1995 and December 1996 and concentrated on each mollusk species, in turn, as shown in Table 1.

TABLE 1.
Scheduling of trials and minimum depuration temperatures.

Species	Trials Period	No. Trials	Average Meat Yields (%)	Minimum Depuration Temperature Prescribed in the UK
Mussels (<i>Mytilus edulis</i>)	Nov 95–Feb 96	60	25.0	5°C
Pacific oysters (<i>Crassostrea gigas</i>)	Mar 96–June 96	60	5.8	8°C
Manila clams (<i>Tapes philippinarum</i>)	July 96	48	18.9	Not specified
Cockles (<i>Cerastoderma edulis</i>)	Oct 96–Nov 96	49	21.6	7°C
Native oysters (<i>Ostrea edulis</i>)	Nov 96–Dec 96	55	10.3	5°C

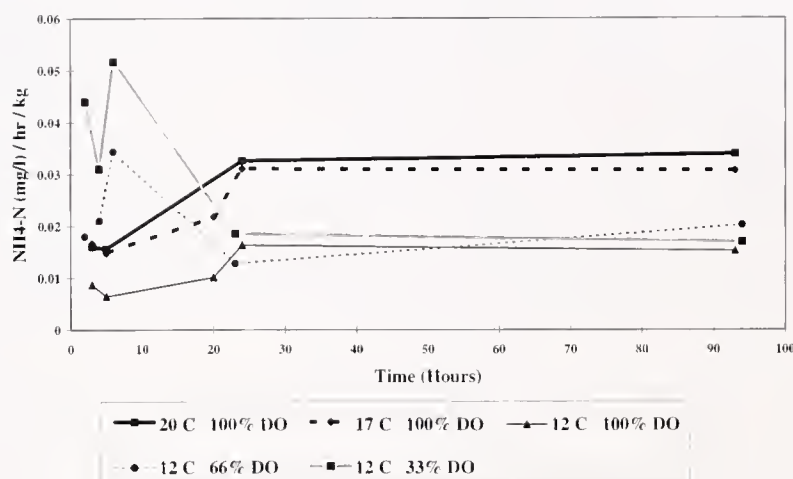


Figure 2. Ammonia excretion rate of mussels per hour against immersion time.

RESULTS

Investigation of Ammonia Excretion as an Indicator of Mollusk Activity

In previous trials investigating the re-use of seawater (Allen 1950, Wilson 1989, Boulter and Denton 1991), the ammonia concentration in the water had been measured before and after depuration. This was done primarily to monitor the cumulative effect of water re-use, there being concern that beyond a certain level of ammonia concentration, mollusk activity would be inhibited. However, experience has shown that when mollusks are re-immersed in a tank after dry storage, their initial level of activity can vary considerably. If starved of oxygen they can initially be very active, whereas, if subject to physical or thermal shock, they can exhibit little sign of activity at all. It was, therefore, thought prudent to measure ammonia excretion after having first given the mollusks time to settle down to the particular experimental conditions.

An initial series of trials was carried out with mussels, under varying conditions of seawater temperature and DO saturation. Ammonia excretion rates per hour (as ppm NH_4N) were measured during the first few hours of re-immersion and subsequently at 17 to 24 hours and 92 to 95 hours. The results are shown in Figure 2. The results show that during the first few hours of re-immersion there is a considerable variation in ammonia excretion rate. This

seems to stabilize after about 20 hours and remains consistent thereafter. On the basis of these trials, it was decided that ammonia samples would be taken after mollusks have been re-immersed for 24 hours.

Comparison of Mollusk Activity Against Temperature in Simulated Depuration Conditions

The results are shown in Figures 3 to 6. Ammonia excretion rates against temperature, shown in Figure 3, indicate that Manila clams and cockles exhibit the most rapid increase in excretion rate with rising temperature, although Manila clams do not increase in activity until the temperature is above 7°C. Mussels increase in activity at a slightly slower rate but show a similar pattern to the Manila clams, with activity not starting to rise until the temperature is greater than 9°C. For native and Pacific oysters, ammonia excretion activity was at very low levels but with a slight linear increase with rising temperature. What is most apparent is the large difference between the oyster species and the other three species. There is some narrowing of this gap when the data are corrected for meat yields, as shown in Figure 4, but oyster activity on the basis of ammonia excretion remains much lower than with the other three species.

DO consumption rates against temperature, as shown in Figure 5, clearly show differences between the species. Cockles exhibit a rapid increase in oxygen consumption with temperature, as do

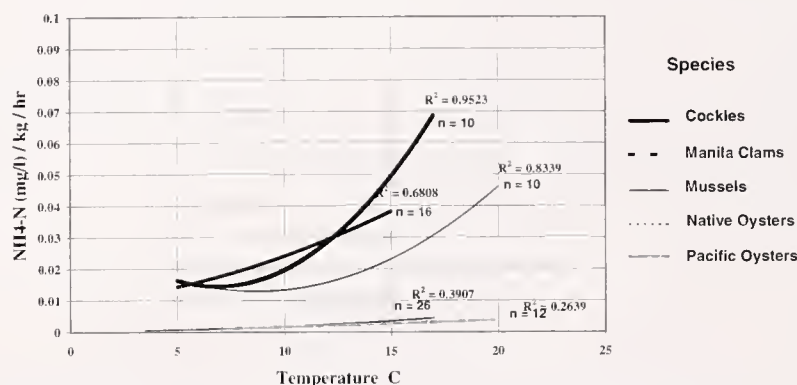


Figure 3. Species comparison of ammonia excretion rates at different temperatures.

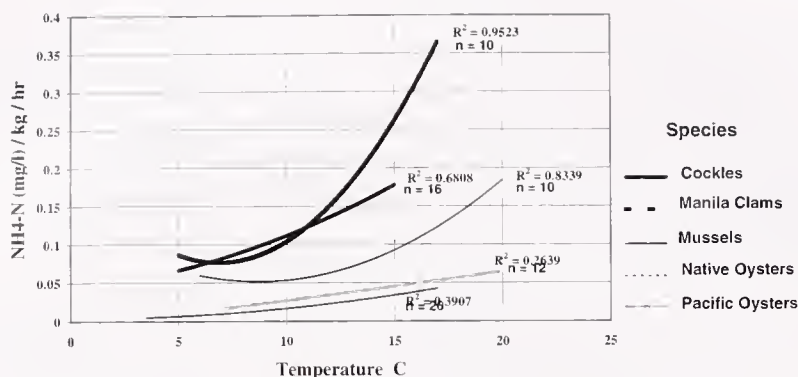


Figure 4. Species comparison of ammonia excretion rates at different temperatures corrected for meat yield.

mussels. Manila clams exhibit low oxygen consumption at the lower temperatures, but consumption rapidly increases as temperature rises. At higher temperatures, all three species show higher oxygen consumption rates than oysters. Native oyster DO consumption is low and shows only a slight increase with temperature. Pacific oyster DO consumption, although not so low, also shows little increase as temperature rises.

Dye reduction data, Figure 6, showed little difference in dye removal rates between the species and little difference with temperatures in the range 5 to 20°C.

DISCUSSION

Effectiveness of the Physiological Response Techniques

Ammonia Excretion

The technique seems to be useful and backs up observation of which species seem to be the most or least active in a depuration tank environment (personal observation and communication with depuration tank operators). The results for the oyster species showed lower significance levels, as can be seen by the R^2 values for the trend lines, (see Figs. 3 and 4), indicating that there was more variance in ammonia excretion activity with these less active oyster species. Notwithstanding this, the results obtained compared quite well with those from the monitoring of DO levels. An advantage of the ammonia excretion technique was that it was generally run over 42 hours, which meant that the results related

well to the depuration process. Unfortunately, numerous trials would be required for the technique to give any meaningful results, which can be time consuming and costly. However, as a technique for measuring the physiological response of mollusks in varying immersed conditions, the measurement of ammonia excretion proved very useful. The information obtained could not have been readily achieved by bacteriological analysis.

Dissolved Oxygen Consumption

This technique was useful, because it could usually be run over only a few hours. The rate of reduction of DO at a particular temperature giving a good indication of mollusk activity rate. Oxygen consumption measured over 1 hour was a useful parallel to the measurement of ammonia excretion, giving some confirmatory and some further information on the physiological response of mollusks to varying conditions. In many cases, this technique was used following a particular ammonia excretion trial and so enabled direct comparison/confirmation.

Dye Removal

Although based on an already established method, this technique did not prove entirely satisfactory for these trials. It is thought that being held in a simulated depuration environment, hence a no food environment, the shellfish reacted to the presence of the dye in the water and took it to be a food source, leading to a fairly standard filtration rate, regardless of temperature or spe-

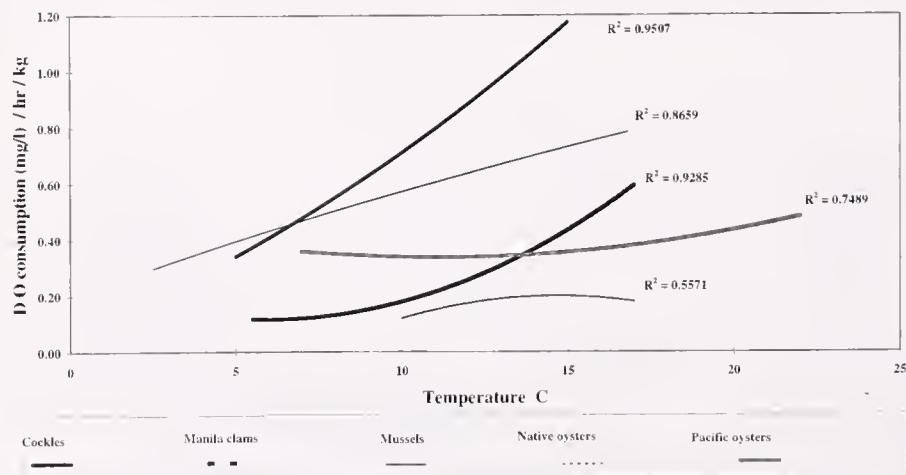


Figure 5. Species comparison of dissolved oxygen consumption rates over 1 hour, at different temperatures.

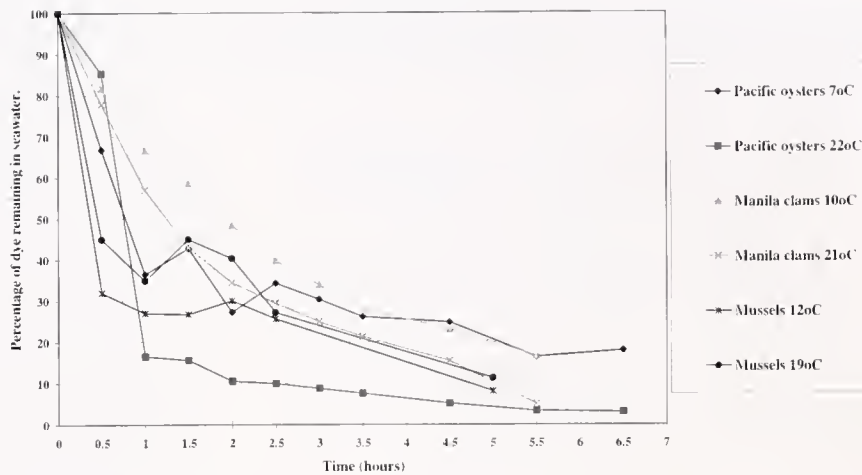


Figure 6. Comparison of dye removal rates by three species at different temperatures.

cies. Dye trials have previously proved useful for determining the point of shellfish inactivity, such as when low temperature or salinity thresholds are being identified. The technique also suffers from the practical disadvantage that the dye must be removed from the trials equipment after each use.

Relevance of this Work to Depuration Systems

The current UK stipulations for minimum depuration temperature (Table 1) are largely based on observation of whether or not the mollusks are functioning, rather than measurement of the level at which they are functioning. These results show that by operating at a water temperature of 15°C rather than the stipulated minimum temperatures, the activity of the mollusk species studied would be at least double that at the stipulated minimum temperatures.

There is concern about the generally low level of activity of both species of oysters together with the associated facts that they are often eaten without cooking and are implicated in the majority

of recorded incidences of food poisoning caused by bivalve mollusks in the UK.

CONCLUSIONS AND RECOMMENDATIONS

Monitoring of ammonia excretion combined with dissolved oxygen consumption proved to be a useful method of obtaining information on the physiological response of bivalve mollusks to varying seawater conditions.

The activity of the mollusks increases with seawater temperatures over the range of temperatures investigated (up to about 20°C). By operating at 15°C, the rate of activity of the mollusks is at least double that at the minimum temperatures currently specified in the UK for depuration system operation.

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HUMAN ENTERIC VIRUSES IN OYSTERS CAUSING A LARGE OUTBREAK OF HUMAN FOOD BORNE INFECTION IN 1996/97

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ABSTRACT During the New Year of 1996/97, more than 350 persons in Denmark became ill from consumption of imported oysters. The main symptoms were vomiting, diarrhea, abdominal pain, and fever, commencing 12 to 48 hrs after consumption. In addition, a number of the diseased persons reported secondary symptoms, such as aching joints, skin numbness, and visual disturbances, commencing 24 h after onset of the primary symptoms. In general, recovery from both the primary and, in particular, the secondary symptoms was slow. Only 3/24 (12%) of the analyzed samples of infected oysters showed an *E. coli* level above the allowed regulatory limit. A small amount of domoic acid was found in 4/9 (44%) of analyzed samples. Small round-structured virus (SRSV) and enterovirus were identified from both oyster and fecal samples, using RT-PCR. Enterovirus isolated from individual fecal samples showed sequence identity of 3 PCR amplicons, suggesting a common source of infection. The identity of the enterovirus is still under investigation. The characteristic clinical symptoms and RT-PCR results implicate SRSVs as the cause of the primary symptoms. However, the cause of the secondary symptoms is currently unclear. Although the detection of enterovirus in both fecal samples and oysters is significant, the incubation period prior to onset of secondary symptoms was not typical of an enterovirus infection. The significance of the domoic acid in relation to the outbreak is unclear.

KEY WORDS: small round-structured viruses, enterovirus, oysters, outbreak, foodborne infection, domoic acid

INTRODUCTION

Human foodborne outbreaks related to consumption of live oysters contaminated with enteric viruses have been reported on numerous occasions (Boher et al. 1995, Morse et al. 1986, Pontefract et al. 1993, Desenclos et al. 1991, Kohn et al. 1995, Chalmers and Mcmillan 1995). Some of the most predominant infective viral agents seems to be Hepatitis A virus and Norwalk virus, belonging to the group of viruses termed small round-structured viruses (SRSV). Because of a lack of adequate detection methodology, the legal requirements regarding the safety for consumption of live oysters are based on the level of fecal coliforms in the oysters (Table 1).

It is generally acknowledged that these requirements are inadequate to ensure public health protection in regard to consumption of live oysters. Currently, however, these requirements are still a legal requirement in the European Union.

THE OUTBREAK

The outbreak occurred nationwide in Denmark during New Year 1996/97, where a total of 356 persons reported illness caused by consumption of imported live oysters. In addition, at the same period of time, 300 people in Sweden took ill from consumption of oysters from the same producer as the infected oysters in Denmark. Batches causing illness in Denmark and Sweden had different expiry dates.

It is believed that approximately 35,000 infected oysters were sold and consumed in Denmark. From the 356 illness reports, it

seemed that the infective doses, on occasions, were as low as a single oyster. It is believed, therefore, that the actual numbers of persons affected was much higher than the number of recorded incidents.

Symptoms

The symptoms reported were of a classical SRSV viral gastroenteritis infection and included diarrhea, vomiting, fever, and headache commencing 12 to 48 h after consumption of oysters. In addition to these initial symptoms, secondary symptoms, such as aching of joints and muscles, dizziness, numbness of skin, face, hands, and legs, visual disturbances, and double vision, were reported to commence 24 h after onset of the primary symptoms. It is estimated from the reported cases that 40% of the infected persons developed secondary symptoms; although only a few reported severe symptoms, such as visual disturbances. In particular, complaints about sore legs were reported.

In general, recovery from the illness was slow. Fatigue and weakness were felt for weeks after the onset of primary symptoms. In particular, persons reporting the secondary symptoms seemed to be recovering slowly. In a few cases, where visual disturbances were recorded, symptoms were reported to last for more than 1 month.

In Sweden, a few people developed Hepatitis A associated with consumption of oysters from the same producer as the oysters causing the Danish outbreak. No Hepatitis A was associated with the affected persons in Denmark.

TABLE 1.

Conditions for production areas for live bivalve mollusks, according to EEC Directive 91/492 on the health conditions for the production and the placing on the market of live bivalve mollusks.

	Category A	Category B	Category C	Bivalves for Direct Consumption
Microbiological criteria for the live bivalve mollusks	<300 Fecal coliforms/100 g or <230 <i>E. coli</i> /100 g	<6,000 Fecal coliforms/100 g or <4,600 <i>E. coli</i> /100 g	<60,000 Fecal coliforms/100 g	As for category A. In addition, no detection of <i>Salmonella</i> in 25-g sample.
Conditions for consumption	Direct consumption	Relaying or purification until requirements for direct consumption are met	Relaying for at least 2 months. Must meet requirements for direct consumption	

Origin of Oysters

Because of inadequate documentation, and even possible fraud with registration documents, it was not possible to trace the infected oysters beyond the producer. Thus, definite origin and handling prior to packaging could not be established. Only the identity of the packer and the expiry dates could be clearly defined.

METHODS

Oysters

The infected oysters came from four consignments with different expiry dates: 25.12.96, 3.1.97, 5.1.97 and 11.1.97. Oysters with expiry date 11.1.97 were not consumed in Denmark, but retained for analysis. Oyster samples with expiry date 25.12.96 were not analyzed for content of virus, because of lack of sample material. Oyster samples were analyzed for bacteriology and marine biotoxins by standard methods and for human enteric viruses by RT-PCR using methods previously described (Lees et al. 1994, Lees et al. 1995).

Stool Samples

Twenty-five stool samples from patients were analyzed by electron microscopy for content of Norwalk virus. In addition, 11 of the 25 stool samples were analyzed for SRSV and enterovirus and poliovirus by RT-PCR. Enterovirus was assayed using a broadly reactive primers and poliovirus using a specific primers.

RESULTS

Oysters

Oyster batches were initially analyzed for enterococcus, *Staphylococcus aureus*, *Salmonella*, *Vibrio* spp., *Listeria monocytogenes*, *Campylobacter*, and *Clostridium botulinum* toxin. No significant amount of any determinant was identified. However, some samples contained a high amount of total aerobic counts at 21°C (>500,000 CFU/g) and a corresponding high level of H₂S-producing bacteria (>10,000 CFU/g), suggesting that the oysters had been subjected to polluted waters. Oyster samples were also analyzed for the marine biotoxins DSP (Diahrrhetic Shellfish Poison) and PSP (Paralytic Shellfish Poison). Both were found to be negative. However, significant results were obtained for *E. coli*, human enteric viruses, and the marine biotoxin domoic acid (Table 2). Although *E. coli* results were well within regulatory limits for the large majority of samples tested, both SRSVs and human enteroviruses were detected in oysters samples. In addition, low levels of domoic acid were found in two of the three batches tested.

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Stool Samples

SRSVs were found in stool samples by both EM and RT-PCR, confirming the etiology of the gastroenteritis in this outbreak. In addition four of 11 stools were positive for human enterovirus (but not poliovirus) by RT-PCR (Table 3). Sequence analysis showed the enterovirus PCR amplicons to have homology with *enterovirus sequence* and also that the three sequences analyzed were identical, suggesting a common source of infection.

DISCUSSION

The results showed that although the vast majority of the analyzed oysters had an *E. coli* level within the allowed regulatory limit, an epidemic still occurred, thus emphasising the inadequacy of using *E. coli* as a suitable indicator of consumer safety of live oysters destined for direct consumption.

In this event, the oysters could not be traced to the original

TABLE 2.
Results of analyses of shellfish samples for *E. coli*, human enteric virus, and domoic acid.

Batch Expiry Date	No. Analyzed Samples with <i>E. coli</i> within Acceptable Limit (cfu/100 g Shellfish)	SRSV in 7.2 g	Enterovirus in 7.2 g	Domoic Acid (mg/kg)
25.12.96	1:<20/100 g	Not analyzed	Not analyzed	Not analyzed
3.1.97	10/12:<230/100 g	Positive	Negative	12.7
5.1.97	6/7:<230/100 g	Positive	Positive	9.8
11.1.97	5/5:<20/100 g	Positive	Positive	Negative

TABLE 3.

Results of analyses of patients stool samples for human enteric viruses.

Method	SRSV	Enterovirus	Poliovirus
RT-PCR	3/11 Positive	4/11 Positive	11/11 Negative
EM	13/25 Positive	—	—

harvesting area, and further handling after harvesting could not be determined. Thus, the sanitary quality of the oysters could only be determined by end product measurements; that is the level of *E. coli* that met the standards. Thus, under current legislation, this event could not have been prevented.

Both SRSV and human enteroviruses were detected in both oysters and stool samples. It is evident that SRSV was responsible for the primary symptoms described, those classical of viral gastroenteritis caused by consumption of infected live oysters and described many times previously (Morse et al. 1986, Pontefract et al. 1993, Kohn et al. 1995, Chalmers and Mcmillan, 1995). The description of the secondary symptoms, more severe than the primary ones, is less typical. The detection of enterovirus contamination of both oysters and stools is intriguing; however, the enterovirus identity has not yet been determined. Sequence analysis

showed the enterovirus amplified from three separate stool samples to have a common identity, suggesting a common source of infection. It is not yet clear whether an enterovirus infection contributed to the range of secondary symptoms seen. Although the detection of enterovirus in both fecal samples and oysters is significant, the incubation period prior to onset of secondary symptoms was not typical of an enterovirus infection.

Domoic acid is known to cause amnesiac shellfish poisoning, with symptoms such as nausea, vomiting, fever, diarrhea 24 h after ingestion, followed by neurological symptoms, confusion, coma, and death 48 h after ingestion. Although this pattern, in some sense, resembles the pattern of symptoms reported in this outbreak, the levels of domoic acid found in the oysters is well below the set regulatory limit of domoic acid of 20 mg/kg shellfish. The significance of the domoic acid in relation to the outbreak is also, therefore, not clear.

It is becoming clear that oysters can be infected with a cocktail of human pathogenic enteric viruses (Sugieda et al. 1996), which can complicate the identification of the causative virus or strain. Clearly, there is a need for better detection methodology for virus to be used in control and regulation. The application of such methods will, to some extent, be complicated by the possibility of contamination of live oysters with a variety of pathogenic viruses.

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DETOXIFYING PACIFIC OYSTERS (*CRASSOSTREA GIGAS*) OF THE NEUROTOXIC SHELLFISH POISON (NSP) PRODUCED BY *GYMNODINIUM BREVE*

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ABSTRACT *Gymnodinium breve* cells were cultured in the laboratory and fed to Pacific oysters at rates of between 10 and 25 million cells per oyster over 24-h periods. Determination of neurotoxic shellfish poisoning (NSP) levels in the shellfish by mouse bioassay revealed initial levels between 25 and 100 mouse units (m.u.) per 100 g drained oyster meat, with larger animals accumulating more toxin per g than smaller ones. The shellfish were detoxified under various conditions in laboratory tanks over 5-day periods using a half-factorial experimental design. The experimental factors were temperature (15 and 20°C), salinity (24 and 33–34‰), filtration (5 µm) versus no filtration, and treatment with ozone (to a redox potential of 350 mV in the shellfish tanks) versus passive ultraviolet light sterilization. Two additional experiments investigated the effect of toxifying oysters over 5-day periods. Significant ($p < .05$) decreases in toxin levels were usually only observed over the first 3 days of the trials. Seven of the eight treatments brought mean toxin levels below the regulatory limit of 20 m.u./100 g within 3 days, regardless of the initial toxin level. However, in four treatments, some individual samples exceeded 20 m.u./100 g after 3 days' detoxification. None of the experimental factors had statistically significant effects ($p > .05$) on detoxification. The implications for commercial detoxification of shellfish from blooms of toxic algae are discussed.

KEY WORDS: detoxification, neurotoxic shellfish poison (NSP), Pacific oyster, *Gymnodinium breve*, depuration

INTRODUCTION

The introduction of Pacific oysters (*Crassostrea gigas*) to New Zealand less than 30 years ago has resulted in the development of an aquaculture industry worth \$NZ 10,000,000 in exports (Anon. 1997). To ensure that oysters contaminated by sewage are not marketed, the industry has developed an effective sanitation program that primarily depends on classification of growing areas and restrictions on harvesting after rainfall. There are also six licensed depuration plants that use ultraviolet light (UV) for water sterilization. However, these are used only occasionally to maintain market access during periods when growing areas are closed because of high rainfall and concern that runoff of animal fecal organisms from surrounding pastoral lands could contaminate shellfish. In the summer of 1992 to 1993, the industry faced a new challenge in providing safe products to the consumer: for the first time, New Zealand shellfish were implicated in cases of shellfish poisoning caused by toxic algae. Surprisingly, the first algal toxin to cause illness from New Zealand oysters turned out to be NSP (neurotoxic shellfish poison), a toxin previously only reported in Florida and other states of the East Coast of the United States. Although the algae causing the New Zealand outbreak were not maintained in culture long enough to be fully identified, they were similar to, but not identical to *Gymnodinium breve* (Chang 1995), the organism that regularly causes closures of shellfish beds in Florida. In response to the initial outbreak, New Zealand adopted a regular shellfish monitoring program for NSP (and other toxins). The New Zealand method of testing for NSP involves an initial screening of shellfish for lipid soluble toxins by a mouse bioassay using an acetone extract. Positive samples are then tested for DSP (diarrhetic shellfish poison) by enzyme-linked immunoassay (ELISA), and NSP is quantified by the APHA method (APHA 1970). A regulatory level for NSP has been set at 20 m.u. per 100

g of drained shellfish meat. There have been no cases of NSP since the initial incident and only occasional closures of shellfish beds because of presumptive NSP-positive shellfish.

The goal of the current work was to develop a procedure that would allow safe harvest and sale of Pacific oysters affected by NSP. Several reports have indicated that *G. breve* cells and NSP toxins are destroyed in an ozone depuration system (Blogoslawski et al. 1973, Blogoslawski et al. 1975, Schneider 1991, Schneider and Rodrick 1995), but no work has been reported on attempting to remove NSP toxins from shellfish. In addition, we have shown that depuration of fecal coliforms from Pacific oysters is effective at temperatures between 15 and 20°C but is less effective at lower temperatures (Buisson et al. 1981, Fletcher et al. 1991). To determine whether levels of NSP toxin in Pacific oysters could be reduced below regulatory levels in a commercially practical time frame, ozone depuration was compared with UV depuration at different temperatures and salinities, with or without filtration of the recycled seawater. Ozone was used because of its reported effectiveness against NSP, and UV was used in the control tanks to prevent the growth of bacteria in the seawater. Salinities and temperatures were chosen to reflect the range likely to be experienced by commercial depuration plants located in the Pacific oyster growing areas of New Zealand. Filtration was included to eliminate any whole algal cells that might be recycling in the tanks.

METHODS

Because initial attempts to isolate and grow species of NSP-producing algae from New Zealand waters failed, we used the Wilson strain of *G. breve* originally isolated in the United States. This strain was supplied by N. R. Towers, AgResearch, Ruakura, New Zealand who received it from C. Tomas, Dept. of Environmental Protection, Florida Marine Research Institute, St. Peters-

burg, FL. The algae were grown in modified Wilson's NH15 medium (Gates and Wilson 1960) for run 1 and in GP medium (Loeblich and Smith 1968) modified to include 85% seawater and 15% distilled water for runs 2 to 6. For runs 1 to 4, the algae were batch fed to 220 Pacific oysters at rates of 10 to 19×10^6 cells.oyster⁻¹ (Table 1), and the oysters were removed after 24 h. For run 5, 30 oysters were fed a batch of 5 million cells/oyster⁻¹ each day for 5 consecutive days, and another 40 oysters were fed 24.5 million cells.oyster⁻¹ in a single batch. Unlike other runs, the latter group of oysters did not remove all the algae from the water. For run 6, 90 oysters were fed a batch of 3.5×10^6 cells.oyster⁻¹ each day for 5 consecutive days.

The experimental detoxification system consisted of two closed systems (Fig. 1): one of four 50-L tanks plumbed in parallel to a temperature-controlled sump with the potential to ozonate the water and the other of a single 50-L tank maintained at the same temperature. Both systems had in-line 5- μ m cartridge filters (FilterPure 5PW10, Contamination Control, Auckland) and ultraviolet lamps (Steriflo 369P, Contamination Control, Auckland) available. The lamp in the unit for tank 5 was covered with aluminium foil for 75% of its length to give a similar exposure for a given flow of water as the other tanks. The tanks were individually aerated using air stones and water flows of 2.5 L/min⁻¹ (3 cycles/h⁻¹) were maintained for each tank in all runs. Ozone could be supplied to tanks 1 to 4 by a corona discharge generator (CD1000, Novozone, Auckland) using oxygen as the source gas. The ozone was supplied to the sump tank by means of a venturi. Water was ozonated to provide a redox potential (redox electrode MC241, Radiometer) of 350 ± 20 mv in the shellfish tanks using an electronic controller. Ozone and ammonia levels in the seawater were measured using Palintest Photometer5000 methods (Anon 1994), and salinity was determined using a refractometer (Atago). NSP levels in the oysters were measured using the APHA method (APHA 1970). Each NSP test was on at least 100 g drained meats from 10 to 12 oysters. This method can detect toxin levels down to about 10 m.u./100 g⁻¹, depending on the size of mice used (18 to 22 g). Where toxicity was observed, but the relevant mice did not die, this was scored at half the minimum detectable level. Crude

lipid levels were determined by the weight of the lipid extracted in the ether extraction during the NSP extraction procedure.

Table 1 shows the experimental parameters for the six runs. The main experiment consisted of four 5-day detoxification runs (runs 1 to 4) making light treatment combinations. The treatment combinations of runs 1 to 4 were in a half-factorial design, using either ozone or ultraviolet sterilization; in-line filtration to 5 μ m or no filtration; water temperatures of 15°C or 20°C; and salinities of 33 to 34‰ (ambient seawater) or 24‰ (c. 70‰ seawater). Runs 5 and 6 compared oysters that had been fed with *G. breve* over 120 h with those fed for 24 h. For the 120 h uptake experiments, the oysters were fed 5 (run 5) or 3.5 (run 6) million cells of *G. breve* per oyster at the beginning of each day for a 5-day period. They were placed in the detoxification tanks 24 h after the last feeding.

Residual Maximum Likelihood (REML) was used to determine standard errors of difference to test whether observed changes in NSP levels during each run were significant. Analysis of variance (ANOVA) on the NSP levels at days 2 and 3 and on the mean NSP levels after day 2 (exclusive) was used to attempt to determine whether any of the factors had a statistically significant effect ($p < .05$) on the outcome of detoxification in the half-factorial experiment.

RESULTS AND DISCUSSION

NSP toxicity levels for the six runs are shown in Figure 2a-d. Detoxification was biphasic, with an initial period of significant reductions in NSP during the first 2 days of detoxification, followed by a period of minimal detoxification. A similar pattern is observed in detoxification of paralytic shellfish poison from some shellfish species (Bricelj and Shumway 1997). With the exception of run 2, tanks 1 to 4, all treatment combinations resulted in an initial decline in NSP levels reaching a minimum by day 3, followed by a period of minimal reductions and some apparent increases in NSP levels. Because of the variability of the data, rates of detoxification could not be meaningfully determined. ANOVA of the final toxicity levels achieved on days 2, 3 or the mean toxicity for days 3 to 5 (Table 1) did not show any of the experi-

TABLE 1.
Experimental conditions and NSP levels for uptake and detoxification of NSP from Pacific oysters.

Run	Oysters		Uptake		Detoxification Conditions						NSP Levels (m.u. 100 g ⁻¹)	
	Weight (SE) ^b (g)	Lipid (SE) ^c (%)	<i>G. breve</i> supplied (10 ⁶ × Cells/Oysters ⁻¹)	Duration (h)	Start Date	Tanks	Water Treatment	Temp (°C)	Salinity (‰)	Filter (μm)	Start (SE) ^d	End (SE) ^e
1	8.11 (0.21)	—	18.77	24	7/12/95	1-4	Ozone	15	24	5	25.4 (2.4)	19.5 (1.2)
1	8.11 (0.21)	—	18.77	24	7/12/95	5	UV	15	33	5	25.4 (2.4)	14.8 (2.3)
2	17.83 (0.42)	5.46 (0.19)	12.10	24	30/6/96	1-4	Ozone	20	34	5	49.8 (1.9)	20.6 (4.6)
2	17.83 (0.42)	5.46 (0.19)	12.10	24	30/6/96	5	UV	20	24	5	49.8 (1.9)	18.6 (4.7)
3	12.02 (0.17)	3.78 (0.12)	10.45	24	28/8/96	1-4	Ozone	15	34	—	31.1 (2.1)	14.6 (1.3)
3	12.02 (0.17)	3.78 (0.12)	10.45	24	28/8/96	5	UV	15	24	—	31.1 (2.1)	12.9 (1.8)
4	12.60 (0.24)	3.86 (0.13)	10.79	24	1/10/96	1-4	Ozone	20	24	—	38.3 (3.7)	13.3 (3.0)
4	12.60 (0.24)	3.86 (0.13)	10.79	24	1/10/96	5	UV	20	33	—	38.3 (3.7)	16.8 (5.2)
5	14.49 (0.23)	3.92 (0.25)	5 × 5.00	120	9/11/96	1-3	Ozone	20	24	5	60.0 (—)	—
5	14.49 (0.23)	3.92 (0.25)	24.50	24	9/11/96	4	Ozone	20	24	5	101.9 (—)	18.9 (—)
6	12.11 (0.30)	1.98 (0.14)	5 × 3.50	120	4/2/97	5	UV	15	24	—	24.2 (2.4)	19.3 (3.15)

^a Mean drained wet weight of soft tissues for all oysters in the run.

^b (SE) Figures in parentheses are the standard errors of the means.

^c Mean lipid levels for all oysters in the run expressed as a percentage of the drained wet weight of soft tissue.

^d Mean NSP level of the 1 to 2 samples taken at day 0 for the run.

^e Mean NSP levels of all samples taken from days 3, 4, and 5.

Experimental Detoxification System

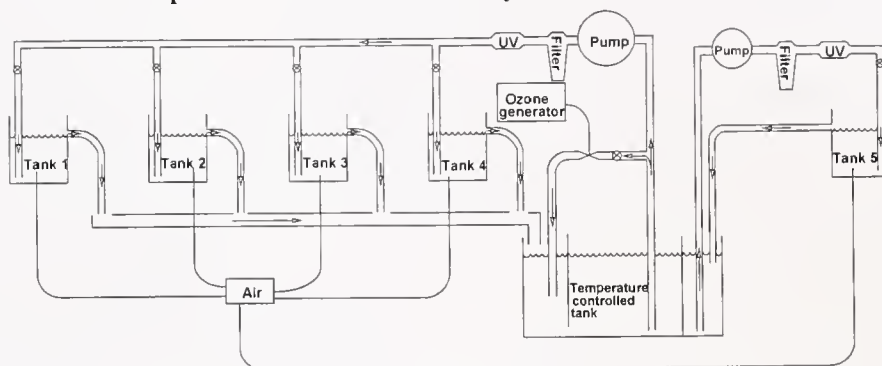


Figure 1. Schematic layout of experimental detoxification system.

mental factors to have any statistically significant ($p < .05$) effect on the outcome. However, the temperature factor was badly confounded with the oyster weight and the starting level of NSP. There was no evidence that ozone improved detoxification over the control using UV light. Averaging the results of samples taken during the detoxification period of minimal change (Table 1, last column) showed that, with the exception of run 2, tanks 1 to 4, all treatment combinations gave mean values below the 20 m.u. 100 g^{-1} regulatory limit. However, current regulations require all

samples to be less than 20 m.u. 100 g^{-1} , and only treatment combinations from runs 1 and 3 achieved this.

Run 5 (Fig. 2f) showed that oysters rapidly reduced NSP levels to near the regulatory limit, regardless of whether the toxin had accumulated over 1 or 5 days. After 2 days of detoxification, there was minimal difference in toxin levels between the two batches of oysters.

In Run 6 (Fig. 2e) the oysters fed 3,500,000 cells *G. breve* per day for 5 days only had mean toxin levels of 24 m.u. 100 g^{-1} 100

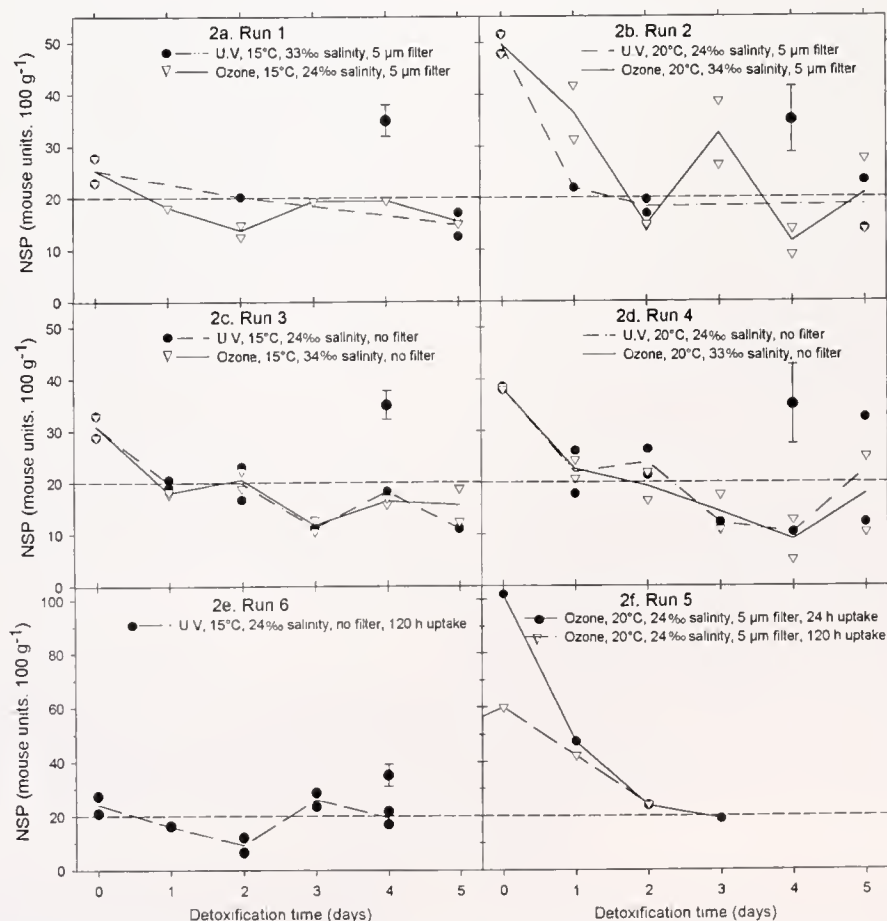


Figure 2. Detoxification progress in runs 1 to 6. Each point represents one NSP analysis of a pooled sample of 10 to 12 oysters. Lines follow the mean NSP levels for a treatment combination. Error bars represent the average standard error of difference for each run as calculated by REMI.

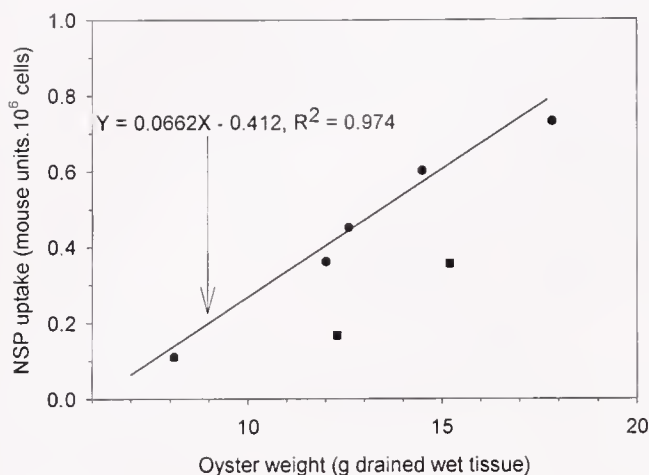


Figure 3. Effect of oyster weight on the rate of uptake of NSP toxin normalised for oyster weights (mouse units per g of oyster flesh over million cells of *G. breve* supplied per g of oyster flesh). ●—24-h accumulation; ■—5-day accumulation. Regression line is for 24 h accumulation only.

g^{-1} . This level showed minimal declines during 4 days of detoxification, suggesting that the second phase of detoxification had already been entered.

Although oysters from different runs had different initial toxin levels ranging between 25.4 and 101.9 m.u. 100 g^{-1} , there were minimal differences between the oyster toxicities at the end of each trial. We were intending to begin all runs with oysters of similar levels of NSP but had difficulty in consistently producing batches of oysters with the same NSP levels. Subsequent analysis of results showed a very clear relationship between toxin levels achieved and oyster tissue weight. Regression analysis of toxin uptake for oysters fed over a 24-h period (i.e., runs 1 to 4, tanks 1 to 3 in Table 1) is presented in Figure 3. For a given level of algae fed per gram of oyster meat, larger oysters absorbed more toxin per gram than smaller ones. Where toxin was accumulated over 120 h, lower rates of toxin uptake were observed, but there was a similar trend with higher toxin accumulation per g by the heavier animals. This trend is counterintuitive, because we would expect smaller shellfish to have higher metabolic rates, and furthermore, that the higher ratio of gill surface area to body weight in smaller animals would result in more efficient filtering on a per g basis. Other workers with other toxins have found that smaller animals accumulate more toxin than larger (Bricelj and Shumway 1997). Those experiments were generally carried out by supplying more toxic algae to the shellfish than they could consume and with final sampling immediately after feeding stopped. In the current work, the shellfish received a batch of algae that was all consumed, and the toxin test was carried out at a fixed time after feeding commenced. Smaller shellfish may have been more efficient at depurating some of the toxin by 24 h when we took the samples. In the wild, either scenario could occur, depending on the distribution

of algae in the water column and on the timing of shellfish harvesting.

Neither the level of lipids in the tissue nor the season (Pacific oysters in New Zealand typically spawn in December or January) seemed to have any effect on the uptake of NSP.

Ammonia levels in the tanks increased at similar rates during all runs at a given temperature. Mean levels of ammonia in the seawater during runs at 15°C on days 0, 1, 2, 3, 4, and 5 were 0.10, 0.96, 1.96, 1.94, 2.75, and 4.01 mg/L^{-1} respectively; whereas, those for runs at 20°C were 0.02, 1.90, 2.25, 5.02, 5.50, and 10.17 mg/L^{-1} , respectively. Although shellfish seemed to be pumping throughout the runs, it is possible that deteriorating water quality was responsible for toxin levels during detoxification remaining relatively constant from day 3 onward. The higher levels of ammonia observed at 20°C could also contribute to the higher SEDs in the runs at 20°C (Fig. 2b, d). Ozone levels in the seawater measured by the Palintest kit averaged 0.12 mg/L^{-1} over all the ozone runs.

CONCLUSIONS

The experimental results indicate that detoxification of oysters containing NSP occurs rapidly under a variety of conditions. That none of the experimental factors had any effect on the final toxin levels suggests that oysters will detoxify regardless of the tested environmental conditions once they are placed in an environment free from toxic algae. Ozone or UV sterilization would only be needed to ensure that numbers of bacterial pathogens did not increase in the tanks and to inactivate any live cells of toxic algae that may be present. However, although the oysters were able to detoxify to mean levels below the regulatory level, the presence of some samples above $20 \text{ m.u. } 100 \text{ g}^{-1}$ means that the process is not yet commercially viable. Further research is being carried out to determine why toxicity levels did not decline after the first 3 days of depuration and to determine whether the effectiveness of the detoxification process can be improved.

If the result showing that larger oysters accumulate more toxin than smaller ones is replicated in the wild, it has substantial implications for regulatory monitoring programs based on testing shellfish flesh for toxicity. If a sample of small oysters gave a low level of NSP, an area could be cleared for harvesting, although large oysters from the same area may still contain substantial amounts of toxin. Work should be carried out on the relative toxicity of large and small animals affected by a toxic incident. If the result is similar to that found in the current study, monitoring programs must take account of this and either sample only the largest animals that are likely to be harvested from an area or only certify the area open for harvesting shellfish up to the size of the animal tested.

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APPLICATION OF THE MICROBIOLOGICAL ASPECTS OF SHELLFISH DIRECTIVE 91/492/EEC IN SCOTLAND

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ABSTRACT EC Shellfish Directive 91/492/EEC lays down health conditions for the production and placing on the market of live mollusks. Under this directive and associated UK legislation, one of the responsibilities of the competent authority is to determine the boundaries of bivalve mollusk production areas and to classify those according to the degree of contamination using bacterial fecal indicators. This paper describes the establishment of the program in Scotland, the classifications issued, research into improving the methodology for *E. coli* analysis, and aspects of the directive for which further development is suggested.

INTRODUCTION

In 1991, the European Union issued a directive that laid down health conditions for the production and placing on the market of live bivalve mollusks (91/492/EEC). This was subsequently incorporated into the UK's Food Safety (Live Bivalve Mollusk and Other Shellfish) Regulations (1992). The directive covers all aspects of mollusk production and aims to "bring about competition on equal terms within member states while ensuring quality products for the consumer" (Anon. 91/492/EEC). To meet these aims it defines criteria relating to preharvest, harvesting, and the end product standard. It also stipulates required standards for transportation, packaging, and depuration of shellfish, and building standards of shellfish premises. The competent authority, which, in this case, is the Scottish Office, is responsible for defining the location and boundary of shellfish production areas and categorizing these sites in accordance with the extent of fecal contamination, determined by the quantity of fecal coliforms *E. coli* in shellfish flesh, measured using a five-tube, three-dilution most probable number (MPN).

Bivalve mollusk fisheries in Scotland consists of both aquaculture and wild fisheries, its value, and the species harvested are listed on Table 1. Mollusks for human consumption can only be harvested from classified sites. Wild caught Pectinids are excluded from this requirement, but all mollusks, including Pectinids and gastropods, must meet the Directive's end-product standard, which includes the absence of *Salmonella* spp., absence of diarrhetic shellfish poisons (DSPs), and less than 80 µg/100 g of paralytic shellfish poisons (PSPs) (Anon. 91/492/EEC).

At the outset of the program, the Scottish Office Fisheries Department determined the most appropriate way to implement the directive to comply with the regulations. One immediate task was to define harvesting areas and to establish sampling procedures and test facilities for fecal coliform analysis. Initially, 125 sites were identified based on known information on registered aquaculture sites and natural beds. Sample collection was devolved to local authorities with Environmental Health Officers collecting samples on a monthly basis for transportation via air, land, and sea to the Fisheries Research Service, Marine Laboratory in Aberdeen, Scotland. Environmental Health Officers were issued with a collection protocol, cool boxes containing ice packs, a thermometer, universals, and forms to record such sampling details as site location and water temperature. The program has now been operational for 6 years, the outcome of which, along with some details of research on the bacteriological methodology are described below.

RESULTS FROM THE SCOTTISH MONITORING PROGRAM FOR THE CLASSIFICATION OF SHELLFISH HARVESTING SITES

Shellfish sites are classified in accordance with levels of fecal bacteria present in shellfish flesh, and the subsequent fate of shellfish after harvest depends upon this classification (Table 2). The directive gives fecal coliform levels for each classification category (Table 2); however, because thermotolerant lactose-fermenting species of *Klebsiella*, *Enterobacter*, and *Citrobacter*, not of fecal origin, can occur in marine waters (Gleeson and Gray 1997), only *Escherichia coli* levels are reported in Scotland. The number of shellfish analysed for *E. coli* and the number of sites classified in the last 5 years are shown in Figure 1. The number of samples processed in recent years has decreased (Fig. 1a) because of a reduction of sampling to quarterly intervals at sites where a 3-year dataset had been accumulated and where no direct source of sewage input was evident. The number of sites classified has increased to 175 (Fig. 1b). This is expected to fluctuate as sites close and others start production. A list of these classified sites is issued annually by the Scottish Office of Agriculture, Environment, and Fisheries Department (SOAEFD).

Because the bulk of harvesting sites occur in areas of low population, where no direct sewage input is apparent, the majority of areas are classified as A (Fig. 2); however, a surprising number were categorized as B (Fig. 2). It is suspected that nonpoint sources such as ineffective septic tanks, farm run-off, seabirds, and marine mammals may be the source of contamination in these areas. In other instances, higher levels of contamination occur intermittently and tend to be seasonal. In these areas, split classifications (A/B) were issued. Figure 3 shows *E. coli* levels over a 5-year period at a seasonally classified site. Low levels of contamination occur during the January to May period, when the site is listed as A; hence, the mollusks may go directly to market. However, shellfish must be depurated before human consumption if harvested in the remaining months (Table 2).

As a result of the occurrence of B-classified harvesting areas, bivalve mollusks depuration systems using UV-light sterilization were installed in several areas and by the end of 1996, 16 approved systems were in place (G. Howard, pers. comm.).

The classification of seven sites as C was not unexpected, because they occur in areas of known sewage input. In this category, the directive allows the shellfish to be relayed for a period of 2 months to meet category A or B requirements (Table 2). This practice in itself may, however, pose problems. If diseased shell-

TABLE 1.
Value of bivalve molluscs landed in Scotland in 1996.

Shellfish Type	Cultivated		Wild	
	Numbers Landed	value (£)	Numbers Landed (tons)	value (£)
Pacific oyster	2,800,000	518,000	—	—
Native oyster	96,000	48,000	2	4,000
Scallops	302,000	151,000	9,539	15,639,000
Queens	1,271,000	63,550	1,896	1,029,000
Mussels	1072 (tons)	991,600	927	171,000
Cockles	—	—	86	43,000
Razor fish	—	—	58	122,000
Clams (various)	—	—	8	4,000

Data for cultivated shellfish adapted from Fraser 1996; data for wild landings obtained from the Scientific Database, Fisheries Research Services, Aberdeen, UK and based on landings by UK registered vessels. The value of cultivated shellfish was calculated from the average of the minimum and maximum price throughout the year.

fish are transferred between areas, the potential exists for transferring pathogenic agents between sites. Similarly, if causative organisms of paralytic shellfish poisons are present, transfer of shellfish between sites has the potential to spread viable cysts of species such as *Alexandrium tamarense*, thereby introducing toxic organisms to previously uncontaminated shellfish. Although shellfish disease is not a significant problem in Scottish waters, *A. tamarense* and other potentially toxic phytoplankton have been detected (Kelly and Macdonald 1996) and pose a potential threat when relaying shellfish. As a consequence, only one relaying site is currently approved in Scotland.

METHOD DEVELOPMENT

The directive states that the procedure for determining levels of fecal coliforms/*E. coli* should be a 5-tube, three dilution MPN, or any other bacteriological procedure shown to be of equivalent accuracy (91/492/EEC). In the absence of further guidance, the precise methodology was defined by a working group for use throughout the United Kingdom. This is a two-stage test, involving

TABLE 2.
Bacterial levels for the classification of shellfish harvesting sites and the treatments for each category (EC 91/492/EEC).

Category	Level of <i>E. coli</i> / Fecal Coliforms	Treatment
A	<230 <i>E. coli</i> /100 g flesh or <300 fecal coliforms/100 g flesh	May go directly for human consumption if end product standard is met.
B	<4,600 <i>E. coli</i> /100 g flesh or <6,000 fecal coliforms/100 g flesh in 90% samples	Must be depurated, heat treated, or relayed to meet Category A requirements.
C	<60,000 fecal coliforms/100 g flesh	Must be relaid for at least 2 months to meet Category A or B requirements.
D	>60,000 fecal coliforms/100 g flesh	Unsuitable for production

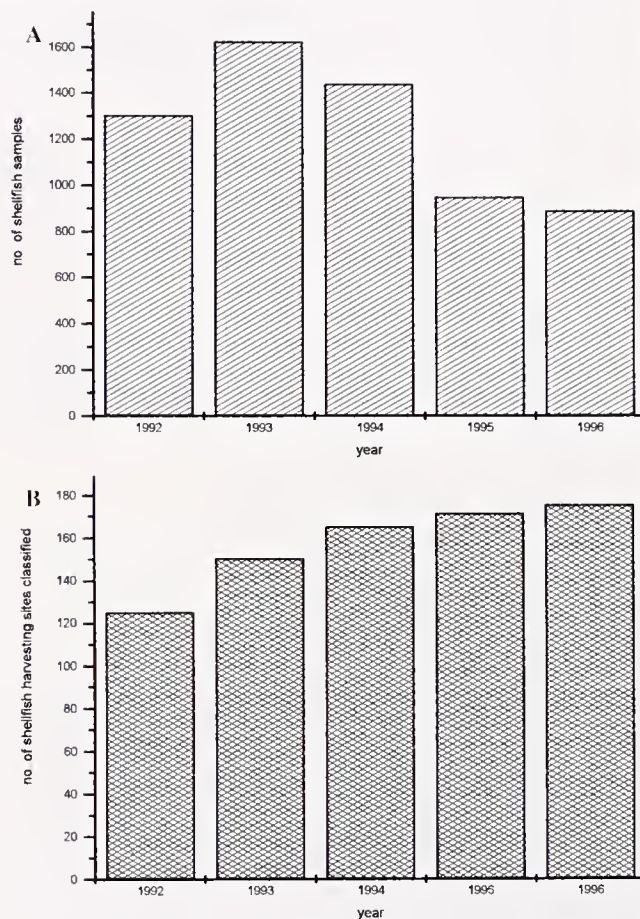


Figure 1a). Number of shellfish analyzed for *E. coli* in the Scottish monitoring program over a 5-year period. Figure 1b). Number of shellfish harvesting sites classified in the Scottish monitoring program over a 5-year period.

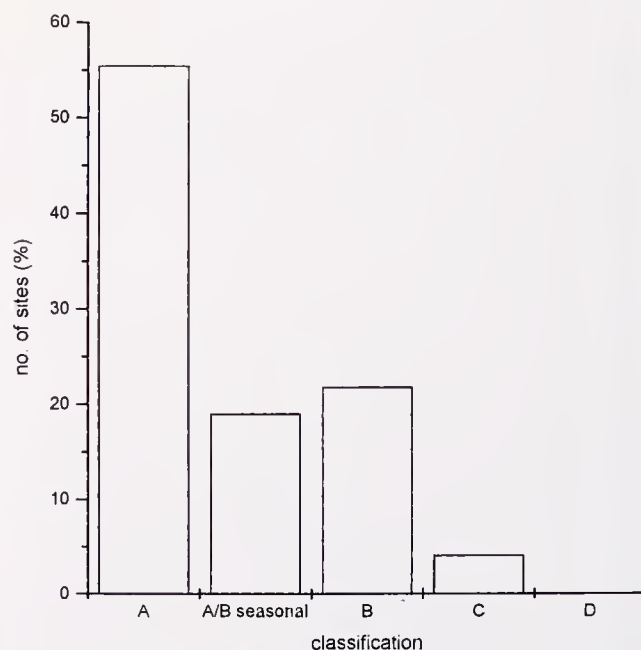


Figure 2. Number of shellfish harvesting sites allocated to each classification category.

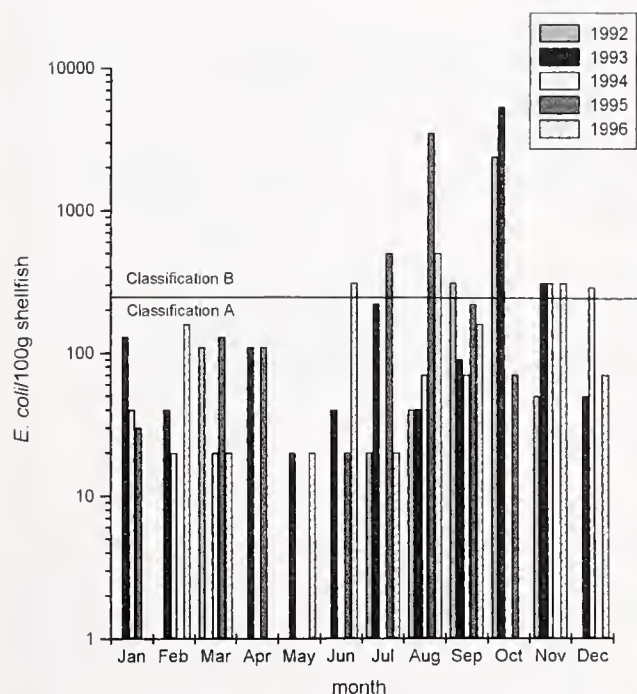


Figure 3. Variation in *E. coli* levels over a 5-year period at a seasonally classified site.

a resuscitation step to recover sublethally injured fecal bacteria (West and Coleman 1986), followed by detection of fecal coliforms based on the production of gas in brilliant green bile broth (BGBB) at 44°C. The presence of *E. coli* is confirmed on the production of indole in tryptone water, again at 44°C (MAFF et al., Working Group, 1992). The *E. coli* confirmatory stage of the procedure uses 30 test tubes and 15 Durham tubes per test and is expensive in terms of labor, media, and consumables. For these reasons, and also in an attempt to simplify the method, the feasibility of using agars containing chromogenic substrates for the detection of β -glucuronidase (β -GUR) were evaluated. This enzyme is highly specific for *E. coli* and reportedly is produced by 95 to 97% of strains (Kilian and Bulow 1976, Perez et al. 1986). The first formula assessed contained a chromogenic substrate named X-glucuronide, and a second substrate Salmon-gal, for the detection of galactosidase activity in coliforms (Chromocult, Merck). The production of these two enzymes by *E. coli* resulted in the formation of purple colonies (Fig. 4).

The second medium contained only one substrate G-glucuronide, which caused *E. coli* to grow as blue/green colonies (Tryptone Bile Glucuronide agar; TBGA; Lab M; Fig. 4). Instead of inoculating presumptive coliforms from MMGM tubes into BGBB and TW tubes, they were streaked onto five segments of one of the chromogenic agars (Fig. 4). The number of segments used, hence the number of agar plates, was dependent on the number of positive presumptive tubes. Therefore, the segments are equivalent to the tubes used in the confirmatory stages of the approved method (Fig. 4).

Oysters and mussels from the samples used in the classification program were analyzed by both the official UK method and the two chromogenic agars and incubated at 37°C and 44°C. Statistical analysis of the data demonstrated that there was no significant difference in the results from either method at both temperatures as compared to the official technique. However, less interference oc-

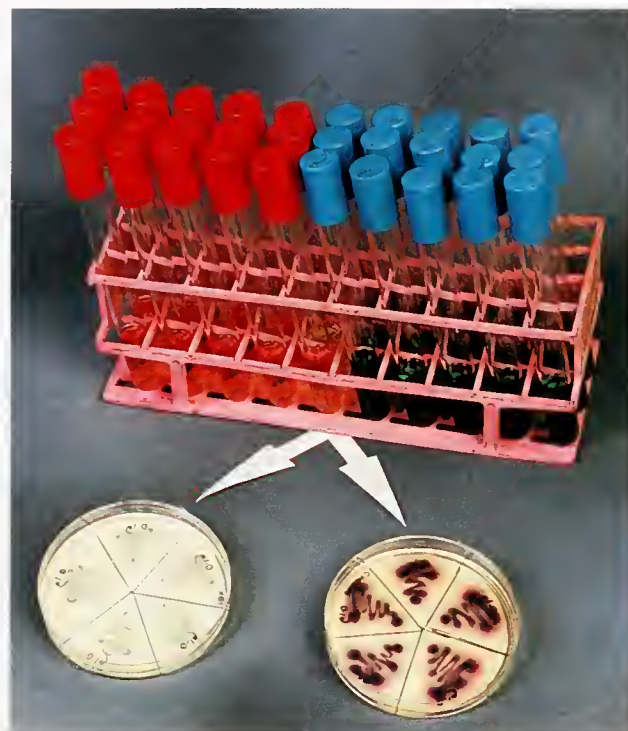


Figure 4. Brilliant green bile broth and tryptone water tubes used in the confirmatory stage of the official UK method compared to the use of either Chromocult or TBGA agar.

curred at 44°C with both chromogenic agars from the growth of other coliform bacteria. Use of these agars substantially reduced the labor cost of sample analysis, and the conclusion of the study was that both are suitable replacements to the confirmatory stage of the UK's official method (Gallacher et al. in prep).

Subsequent to this study, a chromogenic media for the detection of *E. coli* in shellfish has been assessed in a 5-laboratory intercomparison trial, which also concluded that the technique could be used in an adaptation of the UK method (Donovan et al. in press).

CONCLUSION

The classification of existing Scottish shellfish harvesting sites as directed by UK and EU legislation has been achieved. The work is expected to continue as more sites are established and verification of the classified status of existing sites is maintained. However, the EU directive will have to evolve further in several ways before it meets its objective to "bring about competition on equal terms within Member States while ensuring quality products for the consumer" (91/492/EEC).

One aspect to consider is the methodology used to classify areas based on fecal indicators. The option to measure fecal coliforms should be removed, because they may originate from nonfecal sources (Gleeson and Gray 1997). Therefore, within the existing standard, only *E. coli* should be reported.

Several procedures, of varying sensitivity (West and Coleman 1986), are available for the measurement of *E. coli*. This was recognized as a potential problem at the outset of the program within the UK. As a result, a working group devised the current procedure allowing uniformity of testing in Scotland, England, Wales, and Northern Ireland. Although this method may evolve

with the incorporation of chromogenic agars, any changes would require agreement on a UK basis. However, throughout Europe, the methods used vary, which suggests that classification of sites between countries may differ solely because of methodologies.

Another factor is the ineffectiveness of *E. coli* as an indicator of human pathogenic viruses (Jaykus et al. 1994; Lees et al. 1998). Several studies have shown that viral pathogens may be present in shellfish from A-classified sites, and evidence exists that human infection may have resulted in some instances. Even after depuration, viruses may not be effectively removed (Lees et al. 1998). Incorporation of a viral indicator in the form of F+ phage has been suggested (Lees et al. 1998). However, caution must be applied to its use in sites where pollution from animals may be the likely

source of the phage, thereby negating its effectiveness as an indicator of the presence of human viruses.

Additional criteria that must also be addressed by the directive are: specifications on the procedures; frequency and timing of sampling; and establishing the temperature/time criteria for sample analysis after collection. Nevertheless, the EU directive, as it currently exists, is the first stage in ensuring a quality shellfish product throughout Europe.

ACKNOWLEDGMENTS

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MONITORING FOR PARALYTIC SHELLFISH POISONS IN SCOTLAND AND PROGRESS IN RESEARCH TO REPLACE THE USE OF THE MOUSE BIOASSAY

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ABSTRACT The Scottish PSP monitoring program was extended in the early 1990s to take into account the requirements of EC Directive 91/492/EEC and associated UK legislation. This resulted in an increase in the number of samples processed by the AOAC mouse bioassay to quantify the levels of toxin in shellfish. Use of this technique is becoming increasingly unacceptable in many European countries; hence, a research program was instigated to investigate the use of alternative methods. PSP data from the monitoring program and progress on research in the assessment of these alternative methods is presented.

KEY WORDS: PSP, mouse bioassay, cell assay, tissue culture

INTRODUCTION

Paralytic Shellfish Poisons (PSP) are a group of neurotoxins that may accumulate in mollusks, resulting in potential health problems in human consumers (Kao 1993). A monitoring program for their detection has operated in Scotland since 1968 and was increased in scale in 1990, when record levels of toxicity were detected on the Scottish west coast. Subsequently, such monitoring became a legal requirement because of the introduction of EU directive 91/492/EEC, which is enacted in the United Kingdom through "The Food Safety (Live Bivalve Mollusks and Other Shellfish) Regulations" 1992. Samples of bivalve mollusks are collected throughout the year from inshore shellfish aquaculture sites and from offshore scallop fishing grounds. Currently, 43 inshore sites are examined, the testing frequency varies according to the perceived risk of toxicity, based on historical data and the importance of the area to aquaculture. High-risk sites are monitored weekly, and low-risk sites are monitored at 2-week intervals, from April to September. All sites are monitored monthly, from October to March. If toxicity is detected, sampling frequency at affected and adjacent sites is increased, and the number of shellfish species examined is also increased. If the action level of 80 µg STX equivalents/100 g tissue is exceeded, restrictions on fishing and harvesting are imposed. A phytoplankton monitoring program was incorporated in 1996 and has demonstrated that potentially toxic dinoflagellate species are present in Scottish coastal waters (Kelly and MacDonald 1996).

The preferred method for analyzing shellfish for PSP is "the biological testing method," which is generally accepted to mean the mouse bioassay (AOAC 1990). Increasingly, the use of this technique is becoming unacceptable for ethical reasons in a number of countries. For this reason, research was undertaken with the primary aim of evaluating alternative techniques for use in PSP monitoring programs. The research currently focuses on two techniques, the mouse neuroblastoma (MNB) assay and a commercial enzyme-linked immunosorbent assay (ELISA) kit.

The MNB assay uses mouse neuroblastoma cells that express active sodium channels, thereby allowing detection of sodium channel blocking toxins, such as PSP (Gallacher et al. 1993). The cell line arose from a spontaneous tumor in mice and was adapted to *in vitro* culture in 1967 (Klebe and Ruddle 1969). It was used extensively in early studies defining the mode of action of the sodium channel (Catterall and Nirenberg 1973, Catterall 1977). Data from these studies established that exposing MNB cells to

two chemicals, ouabain and veratridine, resulted in an influx of sodium ions, which, if left unchecked, led to cell death. In the presence of toxins that exhibit sodium channel blocking activity, such as saxitoxin (STX), the cells survived. Subsequently the technique was modified from a radioassay to one using morphological observation to determine the physical status of the cells after exposure to the toxins (Kogure et al. 1988).

Further developments of the technique removed the requirement for microscopy, which was laborious and subjective, by incorporating the use of chemicals (Gallacher and Birkbeck 1992, Jellet et al. 1992, Manger et al. 1993), such as neutral red (Gallacher and Birkbeck 1992), which gave a colorimetric reaction and could be quantified using a microtiter plate reader. Because the technique determines total toxicity, regardless of which of the PSP are present, several workers suggested that the assay had the potential to be used to detect PSP in shellfish monitoring programs (Jellet et al. 1992, Gallacher et al. 1993).

The second technique examined was a competitive enzyme immunoassay, the RIDASCREEN Saxitoxin ELISA, which incorporates a polyclonal STX antibody. This paper discusses its use, along with data from the MNB assay and the Scottish PSP monitoring program.

MATERIAL AND METHODS

PSP Monitoring

Shellfish meats were extracted and analyzed by mouse bioassay in accordance with the AOAC procedure (AOAC 1990).

Method Comparison Program

Forty-five mussels (*Mytilus edulis*) and 45 scallops (*Pecten maximus*) from the 1996 monitoring survey were simultaneously tested in spring 1997 by the mouse bioassay (AOAC 1990), the MNB assay (Gallacher and Birkbeck 1992), ELISA kit (Technical Bulletin), and high-performance liquid chromatography (HPLC) (Franco and Fernandez-Vila 1993). The samples, which had been stored at -20°C, were chosen to provide 15 nontoxic samples, 15 containing PSP in the range 40 to 80 µg/100 g, and 15 containing >80 µg/100 g, of each species. All of the techniques were calibrated using STX obtained from the National Research Council, Canada (NRC), and 15 samples were processed per day, over a 3-week period.

HPLC (Franco and Fernandez 1993) and the mouse bioassay (AOAC 1990) were operated in accordance to published procedures on the 90 samples. The MNB assay was used as described in Gallacher and Birkbeck 1992, with the following amendments: the ouabain and veratridine concentrations were 0.1 mM and 0.025 mM, respectively, and eight STX standards were used over the range 0.029 to 14.95 µg/100 g. The plates consisted of two samples, at dilutions 1/40, 1/50, 1/60, and 1/70 in tissue culture media, and eight STX standards, all of which were done in triplicate. Cell controls consisted of six wells containing cells in tissue culture media. Controls to check the performance of ouabain and veratridine consisted of cells, 50 µL of veratridine, 50 µL of ouabain, and 100 µL tissue culture medium. Tissue culture medium contained the same constituents as previously reported, but the fetal calf serum concentrations were increased to 10%.

The relationship between absorbance and concentration of the standard curve was taken to be a four parameter curve of the form $y = \{(a - d)/(1 + (x/c)^b)\} + d$, where y is absorbance (response), x is concentration (i.e., arithmetic dose), a is the 0 dose response (i.e., the response when $x = 0$), d is the infinite dose response (i.e., the response when $x = \infty$), c is the 50% dose response (i.e., the dose resulting in a response halfway between a and d), and b is the slope of the linear portion of the curve.

This curve is the form used in radioimmunoassays and similar work (Rodbard and Hutt 1974). A program, using the statistical computing system GENSTAT, was written to determine the parameters of the standard curve. The concentrations of toxin (x) in a sample of shellfish meat was given as the value corresponding to the observed absorbance (y) obtained from the standard curve. Confidence limits for the estimated concentration were obtained using Fieller's theorem (Fieller 1940).

The ELISA kits (RIDASCREEN) were obtained from Digen

Ltd (Oxford, UK), and the assay was performed in accordance with the manufacturers instructions, with the exception that shellfish extracts were diluted 1:2,000 and 1:5,000 in sample dilution buffer, and STX from the NRC was used.

RESULTS

PSP Monitoring Data

The maximum level of PSP detected in shellfish from Scottish waters fluctuates on a yearly basis (Fig. 1), with the highest level recorded being 6,000 µg STX equivalent/100 g flesh from mussels obtained in 1995 (Fig. 1).

Over several years, a consistent trend has been noted in which the majority of samples tested are negative for PSP, and levels exceeding the closure limit of 80 µg/100 g occur in less than 10% of cases (Fig. 2). However, the unpredictable occurrence of such high levels of PSP in shellfish was demonstrated at one of the Scottish monitoring sites where closure levels occurred in June of 1994, in May the following year, and earlier still, in April of 1996 (Fig. 3).

METHOD EVALUATION PROGRAM

Toxin Profile of Samples Analyzed

Figure 4 demonstrates that the dominant toxins, in the 90 samples were STX, GTX 4, GTX 3, and GTX 2, as determined by

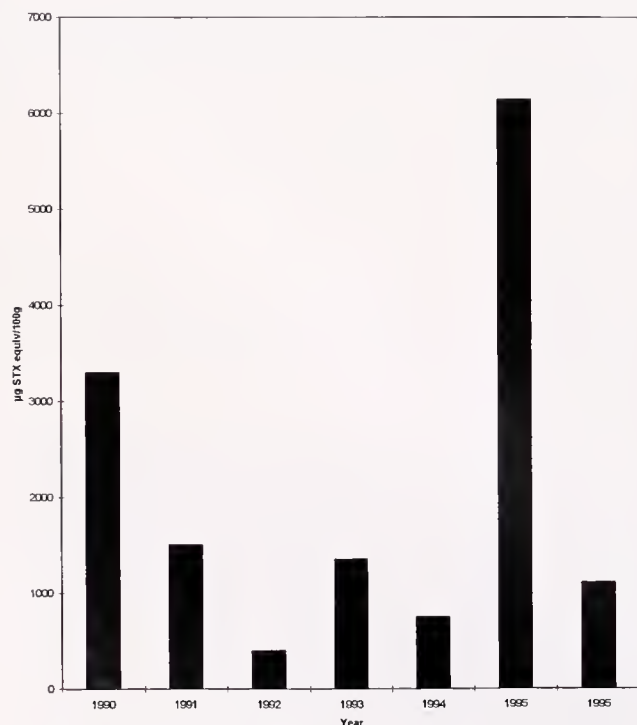


Figure 1. Maximum PSP levels detected in Scottish shellfish over a 7-year period.

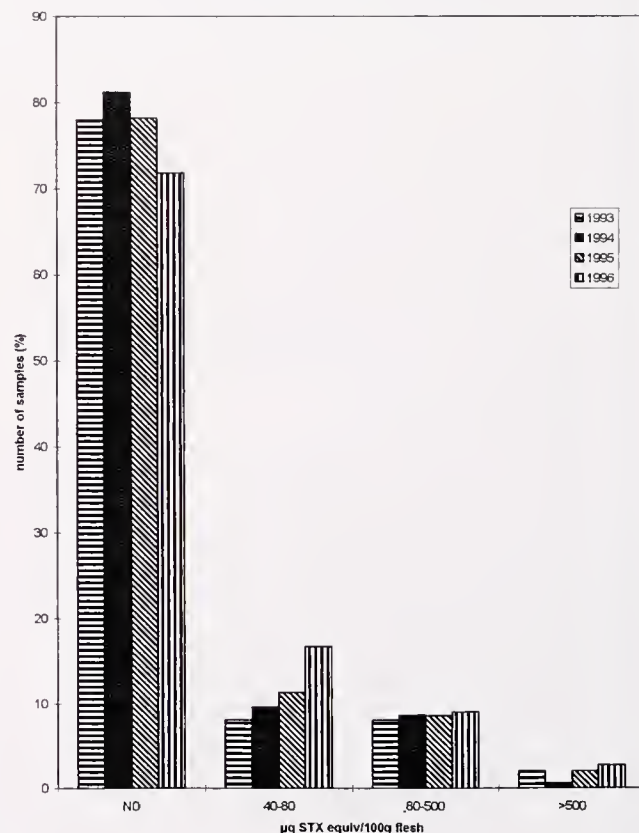


Figure 2. The number of samples from the PSP survey over a 4-year period that are below the 80 µg STX equivalent/100 g flesh closure limit and the number that are above the closure limit.

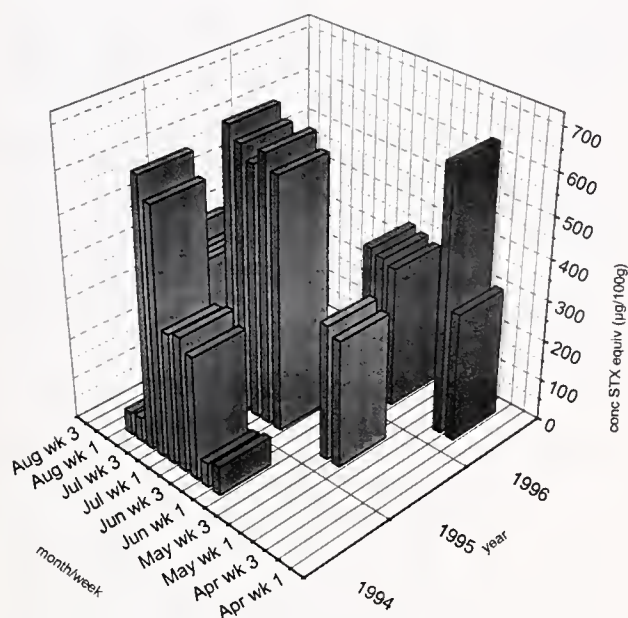


Figure 3. Fluctuation in PSP levels in shellfish on a weekly basis over a 3-year period at a Scottish harvesting site.

HPLC. The presence of GTX 5 and GTX 6 was suspected, but could not be confirmed because of lack of standards.

The MNB Assay

Establishing the Assay

The MNB assay was calibrated at the beginning of the program by determining the concentration of ouabain and veratridine that gave the optimum response to STX. Exposure of the cells to 0.1 mM ouabain and 0.025 mM veratridine produced a sigmoidal dose response curve, where low STX concentrations exhibited little sodium channel blocking activity and high STX concentrations gave cell survival equivalent to the untreated cell controls, hence indicating 100% sodium channel blocking activity (Fig. 5). When ouabain and veratridine levels were too high, the detection limit of

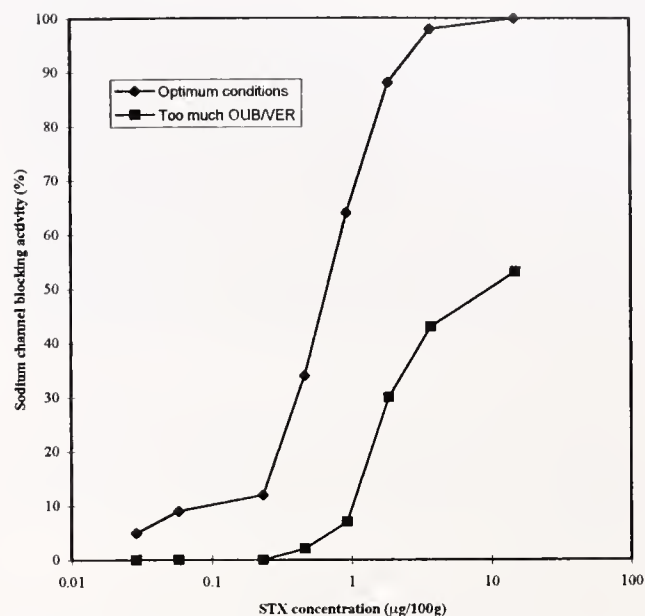


Figure 5. Dose response curve for STX in the MNB assay using optimum and suboptimum concentrations of ouabain and veratridine.

the assay decreased at least five-fold, and the range of sodium channel blocking activity also decreased (Fig. 5).

The STX dose response curve was used to determine dilutions of the sample to be applied to the assay. Because the linear range of the curve was equivalent to 0.234 to 1.869 μg STX/100 g flesh; 9 to 131 μg STX equivalent/100 g of flesh was accurately quantified with the sample dilution range of 1/40 to 1/70. Therefore, a sample that contained 80 μg STX equivalents/100 g of flesh fell into this range if diluted 1/50, 1/60, and 1/70.

Confidence Limits on Sample Values

The estimate of toxicity of any shellfish sample has an associated error based on the uncertainty of the fitted standard STX curve. The precision of an estimated toxicity value was expressed as a 95% confidence interval centered on the estimate (Table 1). For example, for a test sample estimated to contain 62.3 μg STX equivalent/100 g flesh, the 95% confidence limits are 48.7 and 93.0. The estimate itself would trigger increased sampling at the site, but not a harvesting closure, although, the confidence interval

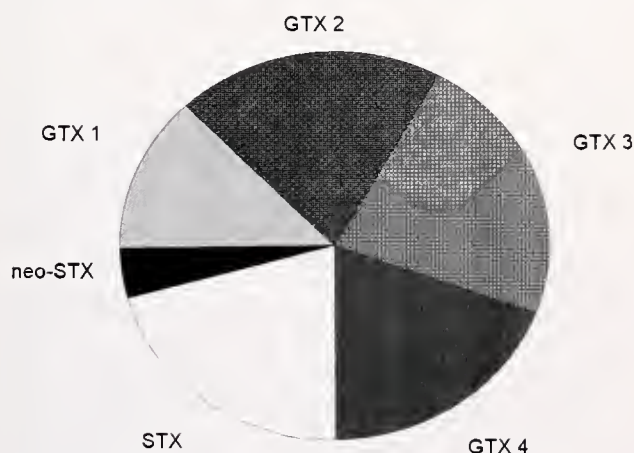


Figure 4. The PSP profile in shellfish used for the method comparison study.

TABLE 1.

Examples of PSP levels in shellfish obtained using the MNB assay and their associated 95% confidence limits.

Estimated Value from the MNB Assay (μg STX equivalents/100 g)	95% Confidence Limits of the Estimated Value (μg STX equivalents/100 g)
35.9	28.5–44.3
41.1	33.1–53.6
54.1	40.9–91.9
62.3	48.7–93.0
94.5	75.6–129.1

shows that the estimate is not significantly different from 80 μg STX equivalent, the closure limit.

Use of the MNB Assay as a Qualitative Screen for PSP in Shellfish

The detection limit for the mouse bioassay used in this study was 23 μg STX equivalents/100 g. In samples deemed negative for PSP by mouse bioassay, the MNB assay either detected PSP at levels lower than the mouse detection limit or in the majority of cases, toxin was absent. Hence, the two assays were in 100% agreement on nontoxic samples. Similarly, in samples defined as toxic by the mouse bioassay, the MNB assay again agreed in 100% of instances.

Use of the ELISA Kit as a Qualitative Screen for PSP in Shellfish

For samples containing <23 μg STX equivalents/100 g by mouse bioassay, the data generated using the ELISA kit agreed in 100% of cases. The ELISA also detected toxicity in all samples deemed to be toxic using the mouse bioassay.

DISCUSSION

In the last 7 years, the Scottish monitoring program for PSP in shellfish has evolved into an effective system that complies with EU directive 91/492/EEC. Highly toxic samples that exceed the level reported to cause illness (van Egmond et al. 1993) have been detected. However, further examination of the data indicates that these levels occur in only approximately 2% of the total number of samples tested (Fig. 2). Nevertheless, the requirements for a monitoring program are amply demonstrated in Figure 3, which shows that precise dates for the occurrence of high levels of PSP cannot be predicted.

As required by legislation, this monitoring program is dependent upon the mouse bioassay (AOAC 1990), which is becoming increasingly unacceptable for ethical reasons. Therefore, a program was instigated that assessed two alternative techniques, the MNB assay and the ELISA RIDISCREEN kit, for their potential to replace the mouse bioassay.

In a study of this type it is essential to identify all sources of variability associated with each procedure and to quantify as many of these as possible, in order to compare their performance accurately. A statistical program, written for the purpose of estimating confidence limits for the alternative techniques, demonstrated that where toxicity was estimated as <80 μg STX equivalents/100 g, the actual value could be above this action level (Table 1). A

similar program for the mouse bioassay is not yet complete, but is expected to show similar, if not higher, ranges in confidence limits. This expectation is attributable to several factors, including reports of up to 1/3 of animals being resistant to a lethal injection and matrix effects causing an approximate 40% reduction in the quantity of toxin detected (Shantz et al. 1958). Although, the mouse bioassay is generally reported to have an associated error of 20% (Shantz et al. 1958), this will fluctuate between samples and must be accurately calculated for method comparison purposes. The statistical analysis of the mouse bioassay is expected to be completed early in 1998, hence comparison of the alternative methods with the mouse bioassay has, for the present, been expressed in a qualitative manner.

The results from the study to date indicate that the MNB assay has potential to be used to screen for positive samples. In the Scottish monitoring program, this would effectively reduce the number of animals used by at least 70% (Fig. 2). Similarly, the data from the ELISA kit demonstrated that this test could also be used as an effective screen. Its advantage over the MNB assay is that results can be obtained on the same day the sample is received. Because the ELISA is purchased as a kit, it is also easier to use, although this advantage has been negated by the commercial availability of the MNB assay in a kit format (Jellett Biotech, Canada). The major disadvantage of the ELISA technique is its limited cross reactivity with neo-STX, GTX 1, and GTX 4 (Usleber et al. 1997), which may pose problems if the assay were used on a quantitative basis. Also, before such a technique could be used with confidence, further work would be required to determine that the PSP profiles found in this study are an accurate representation of the profiles found during the monitoring season. These problems do not exist with the MNB assay, because it mimics the pharmacological action of PSP in mice and measures total toxicity.

In conclusion, both the MNB assay and ELISA show potential for application as screening tests in PSP monitoring programs, and investigations are continuing into their use as fully quantitative techniques.

ACKNOWLEDGMENTS

The authors thank Cheryl Sim, Margaret McCann, and John Turriff for their assistance. This work was funded by Fisheries Research Services and the Ministry of Agriculture, Food, and Fisheries.

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THE MIST™ SHIPABLE CELL BIOASSAY KITS FOR PSP: AN ALTERNATIVE TO THE MOUSE BIOASSAY

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ABSTRACT A shipable cell bioassay kit called MIST™ (Maritime *In Vitro* Shellfish Test) has been developed by Jellett Biotech Ltd. for Paralytic Shellfish Poisoning (PSP). This technology is a cost-effective alternative to the mouse bioassay, and is 20 times more sensitive than the mouse. The kits provide precultured cells and premixed reagents, eliminating the need for tissue culture facilities or expertise and are robust enough to be performed in the field or on a ship. They are available in three formats: fully quantitative, semiquantitative, and a qualitative (yes/no) version, called Mini-MIST™. The shelf life of the quantitative MIST™ Kit is 3 weeks, although this can be extended by freezing the cells to -100°C . The kits have been successfully shipped from Canada to as far away as Australia and New Zealand. The MIST™ technology has been used to determine total PSP toxicity in acid extracts of phytoplankton, lobster hepatopancreas, mussels, scallops, clams, cyanobacteria, and processed food. It has also been used to determine toxicity in phytoplankton samples. Results of parallel trials with the cell bioassay and the mouse bioassay are summarized.

INTRODUCTION

The MIST™ Kit for Paralytic Shellfish Poisoning (PSP) is based on a neuroblastoma cell bioassay method developed and validated by Jellett et al. (1992, 1995). In simplistic terms, the competitive reagents used in the bioassay will destroy the cells unless they are protected by the presence of saxitoxin. This method was itself based on discoveries made earlier by Catterall and Nirenberg (1973) and Kogure et al. (1988), who saw the potential for utilizing the competitive interaction of sodium channel blockers and activators on the channels of neural cells. Concurrently, Jellett et al. (1992), Gallacher et al. (1992), and Manger et al. (1993) developed automated endpoints for the Kogure (1988) method, based, respectively, on direct cell staining, vital stain uptake, and tetrazolium dye reduction. This allowed the use of the cell bioassay for detection and quantitation of PSP toxins. The method, however, still required tissue culture expertise and facilities. Jellett Biotech has succeeded in developing a method to keep the cells in stasis at $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$, while they are shipped and stored, thus giving shelf life and shippability. The cells have been incorporated into a kit format for ease of use.

RESULTS AND DISCUSSION

Kit Formats

The MIST™ kits for PSP have been developed into three formats: quantitative, semiquantitative, and qualitative (yes/no). The fully quantitative version utilizes a neutral red end-point modified from Gallacher et al. (1992), requires a microplate reader, and gives a determination in μg saxitoxin (STX) equivalents ($\text{eg}/100$ g shellfish tissue). Serial dilutions of pure STX and of each of four unknown samples are prepared for each test plate. Aliquots of these dilutions are then applied to the test plate containing cells, followed by aliquots of the competitive reagents. After incubation, the test plates are stained, and the amount of color obtained is proportional to the amount of STX to which the cells have been exposed. The color resulting from each dilution of an unknown sample is then compared to that from the standard curve of pure STX using a microplate reader, thus deriving a toxicity value for

each dilution. The mean of several replicate values derived from each unknown sample is then calculated, giving a fully quantitative determination of total toxicity in that sample.

The limit of detection of the MIST™ kits for PSP is about $2 \mu\text{g}/100$ g, about 20 times more sensitive than the standard mouse bioassay. Because the mouse neuroblastoma cells utilized in the kits are derived from a tumor, they are immortal, and no animals are ever used. These kits can be held at room temperature ($20 \pm 5^{\circ}\text{C}$) for 3 weeks after they are produced. The plates then require 48 hours of preincubation to activate the sodium channels that are the basis of interaction of the cell test.

The semiquantitative version is read by eye without the aid of instrumentation and places the toxicity of the sample extract into one of six ranges: 0–2; 2–10; 10–50; 50–250; 250–1250; 1250 $\mu\text{g}/100$ gm + (Fig. 1). The ranges can be set to detect higher or lower toxicity by altering the dilution scheme used in the preparation of the samples. The qualitative Mini-MIST™ (Fig. 2) indicates color when sample toxicity exceeds a set limit (either 25 or 50 $\mu\text{g}/100$ g in our current test versions) and shows pale or no color when toxicity is below this limit. Both semiquantitative and qualitative versions utilize the original crystal violet end-point (Jellett et al. 1992), which gives more brilliant color for visual determinations, and both have controls incorporated in every plate to ensure that the tests are performed correctly and for comparison purposes. Equipment requirements and costs, therefore, vary, depending on which version of the kit is used.

Applications of the MIST™

All versions of the MIST™ kits for PSP can be utilized with a wide variety of sample types. Because the method detects total toxicity, the AOAC 0.1 N HCl boiling acid extraction method (AOAC 1989) is recommended because of the Proctor enhancement effect. However, milder acetic acid extraction methods used for analytical techniques such as HPLC can also be utilized, bearing in mind that the full toxicity potential of the sample will not be achieved. Various matrices have been successfully utilized in the cell bioassay method, including extracts of whole bivalve, gastropod, and crustacean tissues (Jellett et al. 1992, Ogata et al. 1989, Truman and Lake 1996), scallop digestive glands, and complex

Interpretation of the MIST™ Plate

Purple = PSP; More purple = more PSP

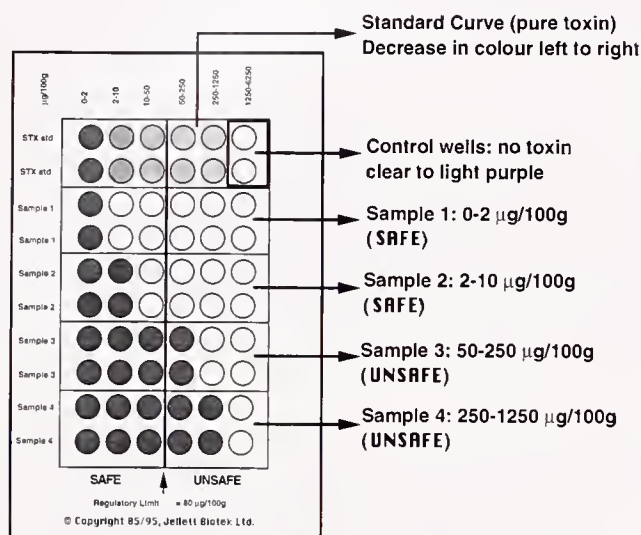


Figure 1. Interpretation of the MIST™ semiquantitative kit for PSP. Darker color means more toxicity.

processed seafood [unpublished], bacterial culture medium (Gallacher, et al. 1992, Komada et al. 1990), and freshwater cyanobacteria [unpublished], as well as various preparations of pure tetrodotoxin and several analogs of saxitoxin (Jellett et al. 1973, Kogure et al. 1988, Sato et al. 1988). The method has also been successfully employed on extracts of phytoplankton concentrated from seawater using net tows or centrifugation, and from culture (Jellett et al. 1992). Extracts of lobster hepatopancreas were found to have cytotoxic affects, and a C18 column cleanup was required to successfully determine PSP toxicity in this matrix type (Jellett et al. 1992). Some extracts show matrix affects at high concentration, but this affect disappears when the extracts are diluted in the standard MIST™ kit sample preparation procedures and does not interfere with determination.

Further Developments of the Kit

The cells have now been successfully frozen and stored at -100 to -140°C , revived, and utilized in the cell bioassay. This storage method is under more rigorous testing now, because it will give users more flexibility in the use of the cells as required. The MIST™ Kits for PSP containing live (not frozen) cells, have now been successfully shipped to Scotland, Denmark, France, Spain, Australia, and New Zealand.

The reporter gene β -galactosidase (Hall et al. 1983) was successfully inserted into the Neuro 2A cells used in the MIST™ kits. Selected clones had the same properties of bioassay performance and shelf life as the nontransfected cells. However, development of the end-point required manipulation more complex than that of the neutral red and crystal violet end-points; therefore, the transfected cells have not yet been incorporated into the MIST™ kits. Other types of reporter genes are currently under investigation in an effort to simplify the kit end-point.

TABLE 1.

Trends of correlation in a parallel trial of cell and mouse bioassays performed with the collaboration of Inspections Directorate of the Department of Fisheries and Oceans in Halifax, Nova Scotia and Health Canada in Ottawa, Ontario.^a

	Cells	Mouse	HPLC
Cells	—	0.961	0.827
Mouse	0.961	—	0.797
HPLC	0.827	0.797	—

^a Extracted from Toxicon 30:1143–1156, 1992.

Similar results were obtained with phytoplankton extracts (Jellett et al. 1992).

Kit Validation

Several parallel trials of the cell bioassay method with other PSP methodologies have been carried out. The first parallel trial between the cell and mouse bioassays was performed on about 100 shellfish tissue extracts (Table 1). The majority (69%) of these samples were below the level of detection ($40 \mu\text{g}/100 \text{ g}$) in the mouse bioassay, and all of these were $<40 \mu\text{g}/100 \text{ g}$ in the cell bioassay. Results from the remaining 31 samples were compared with those from the mouse bioassay and gave a correlation coefficient of 0.993 (Jellett et al. 1992). A subset of 12 toxic samples from this trial were tested by cells, mouse, and HPLC with correlation coefficients >0.90 for cells versus mouse bioassays (Table 1).

The first attempts to ship the cell bioassay in the MIST™ kit formats were made in 1995. The kits were shipped from Halifax, Nova Scotia, Canada to the laboratory of John Hurst in Boothbay Harbor, Maine, USA. Many problems were encountered with cell leakage in transit by air. Rigorous testing in the environmental simulation chamber of the Innovation Corporation of Nova Scotia identified the problem as decompression effects during aircraft

Interpretation of Mini-MIST™ Plate

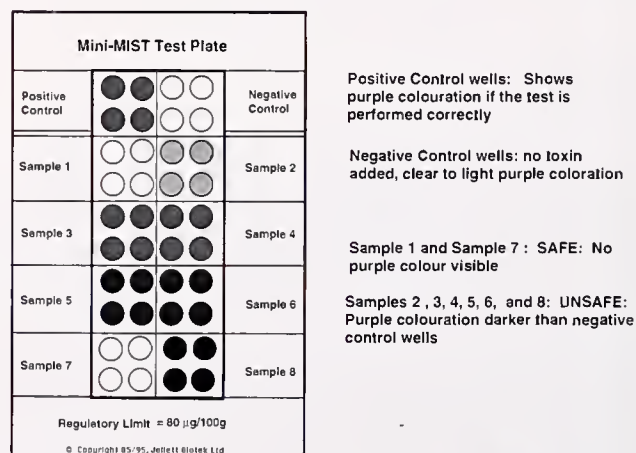


Figure 2. Interpretation of the yes/no qualitative Mini-MIST™ Kit for PSP. This plate has been configured to hold eight samples but can be expanded to hold 18 samples.

takeoff and landing. As a result, a commercial decompression-proof container was identified for subsequent air shipping. In the summer of 1996, these containers were used to ship a small number of quantitative MIST™ kits to another collaborator, the FRS Marine Laboratory in Aberdeen, Scotland. Early shipments experienced some leakage caused by incorrect use of the containers, but some kits arrived safely and produced data. A total of eight samples of shellfish extracts were compared by testing in the fully quantitative MIST™ kits and the mouse bioassay in the Scotland laboratory, and a correlation coefficient of 0.945 was found.

In 1997, a larger parallel trial was completed at the FRS Marine Laboratory in Scotland with 100 shellfish extracts. Staff at the FRS Marine Laboratory tested the extracts in the cell bioassay prepared in their laboratory (Gallacher et al. 1992), in the mouse bioassay, and in all three versions of the MIST™ kits, quantitative, semi-quantitative, and qualitative. Full analysis and publication of these data is awaiting the completion of a statistical program to determine confidence limits for both the cell and mouse bioassays in the Scotland laboratory. However, preliminary comparisons of results showed that good kit performance and shelf life were achieved after shipping from Nova Scotia to Scotland. All samples <40 µg/100 g in the mouse bioassay gave the same results in the MIST™ kits (all versions), underlining the utility of the MIST™

kits for screening of negative samples. Preliminary comparisons of the fully quantitative MIST™ kit results with those from the mouse bioassay showed strong correlation trends (e.g., coefficients of >0.75 for $n = 67$). However, statistical comparisons cannot be made without the availability of data confidence limits, which will be forthcoming when the fully analyzed data are published by the FRS Marine Laboratory. Confidence limits are essential for determination of false positives and negatives around the regulatory limit of 80 µg/100 g.

An AOAC collaborative study is planned for early 1998, where samples of mussel homogenate will contain one of three difference toxicity profiles at one of two different levels (high and low), or high or low levels of saxitoxin standard spiked into the tissue homogenate, or no toxin at all (controls). Samples will be provided in duplicate to test inter- as well as intralab variability, but all samples will be randomly numbered so the participants will not be able to pre-guess the toxicity values. The 14 participating laboratories will each perform testing on the blind samples using the fully quantitative version of the MIST™ Kit for PSP, and additional laboratories will perform the mouse bioassay and chemical analysis on the same samples. Results of the trials will be presented to the scientific community as soon as possible after they are completed.

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VARIATIONS IN THE PSP CONTENTS OF SHELLFISH IN HONG KONG AND THE EASTERN COAST OF SOUTH CHINA SEA

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ABSTRACT Results of a monitoring program revealed that there was a steady level of paralytic shellfish poisoning (PSP) toxins in the marketed shellfish samples in Hong Kong and the eastern coast of South China Sea. The PSP contents varied from 300 $\mu\text{g/kg}$ to 2,500 $\mu\text{g/kg}$. According to high-power liquid chromatography (HPLC) analyses, the major toxins included: CTXs, STX₁, STX₂, GTX₂, GTX₃, and GTX₄. By means of similarity analysis it was discovered that the PSP toxins in shellfishes collected from Macau, Hong Kong, and Mirs Bay were of the highest similarity. The toxins in shellfish samples that were collected from Daya Bay and the eastern coast of South China formed another similarity group. Results of the present study suggested that PSP causative phytoplanktons and the PSP-contaminated shellfish sold in Hong Kong markets were mainly imported from the eastern coast of South China Sea.

KEY WORDS: paralytic shellfish poisoning (PSP), PSP toxin contents, shellfish poisoning in Hong Kong and the South China

INTRODUCTION

Shellfish contaminated with algal toxins are harmful to human beings. Of the various shellfish intoxications, Paralytic Shellfish Poisonings (PSP), Diarrhetic Shellfish Poisonings (DSP), and Amnesic Shellfish Poisonings (ASP) are closely related to harmful phytoplanktons. To reduce health risk, numerous countries, including Hong Kong and China, have implemented monitoring and control programs on harmful phytoplankton and toxin-contaminated shellfish. The physiological mechanisms on toxin production have yet to be concluded. Scientists also found that there were significant variations in toxin levels between different shellfish species, seasons, districts, and even individuals in a monitored shellfish population (Shumway 1997). For such reasons, scientists urged for more comprehensive research and monitoring to understand the spreading mechanisms of harmful toxins as well as the controlling methodology of shellfish poisoning (Smayda and Watt 1995).

MATERIALS AND METHODS

Monthly monitoring of PSP toxins in five species of shellfish, namely Green-lipped mussel (*Perna viridis*), Clam (*Tapes philippinarum*), Bloody cockle (*Anadara crebricostata*), Oyster (*Ostrea* sp.) and Fan-mussel (*Atrina pectinata*), was conducted from January 1993 to December 1996. Samples were collected from seawater of the outer Tolo Harbour in Hong Kong by divers and bought from various shellfish markets in Hong Kong, Shenzhen, and the eastern coast of South China Sea. The origins of the marketed shellfish were recorded according to information provided by the shellfish sellers.

The toxicity levels of shellfish samples were tested by mouse assay (AOAC 1984). The detailed contents of toxins were further analyzed by high-power liquid chromatography (HPLC) according to the methods suggested by Sullivan et al (1987).

Phytoplankton samples were collected at bimonthly intervals at Hong Kong and the eastern coast of South China Sea. Haul-netted (of mesh size 20 μm) samples were obtained and examined under inverted microscopes. Environmental information related to marine water quality in Hong Kong was extracted from the various reports of Environmental Protection Department, Hong Kong SAR Government. The data on shellfish poisonings was kindly provided by the Department of Health, Hong Kong SAR Government.

RESULTS

Seasonal Patterns and Geographic Distribution of PSP

According to monitoring results, it was observed that there was very obvious seasonal pattern on the levels of PSP toxins. The mean PSP concentration in shellfish samples maintained at the levels of 600 $\mu\text{g/kg}$ to 1650 $\mu\text{g/kg}$ (Fig. 1). For concentrations in individual species, the green-lipped mussel, fan-mussel, and bloody cockle accumulated the highest amount of PSP toxin (350–1650 $\mu\text{g/kg}$); whereas, oyster accumulated the lowest amount (200–900 $\mu\text{g/kg}$). The PSP levels were generally highest in late winter to early spring, less high in the winter and late spring, with the lowest in summer and autumn. All the six monitored shellfish species exhibited a similar seasonal pattern; that is, PSP contents in the decreasing order of early spring > winter > summer, and autumn.

It was also noted that the seasonal pattern of PSP toxins in shellfish was closely similar to that of phytoplankton blooms and related to the seasonal destratification of water columns in Hong Kong and the South China Sea (Lam and Ho 1989). Such similarity in seasonal patterns suggested that PSP in the areas was closely associated with the blooms of toxic phytoplanktons and the availability of algal nutrients through seasonal changes in climate.

Harmful Phytoplanktons

The harmful phytoplanktons as recorded in the monitoring program included: *Alexandrium catenella*, *A. tamarensis*, *Gonyaulax polyedra*, and *Chattonella marina* (Table 1). It is noteworthy that most of the harmful phytoplanktons were observed in samples collected at the eastern waters of Hong Kong and the eastern coast of the South China Sea, where the intruded oceanic currents from the South China Sea met the freshwater discharged from the Pearl River. According to a cyst survey, the closer to the eastern coast of South China Sea, the higher the amount of *Alexandrium* cysts being accumulated in the sediment (Fig. 2). The results agreed with those of Ho and Hodgkiss (1993) that *Alexandrium catenella* generally favored waters of high salinity (>30‰) and high pH, which are the major characteristics of oceanic currents.

Contents of PSP Toxins

The contents of PSP toxins in shellfish samples were analyzed by HPLC. It is noted that STX comprised to 40 to 60% by weight

PSP Toxicity in Various Species of Bivalves in Hong Kong Market

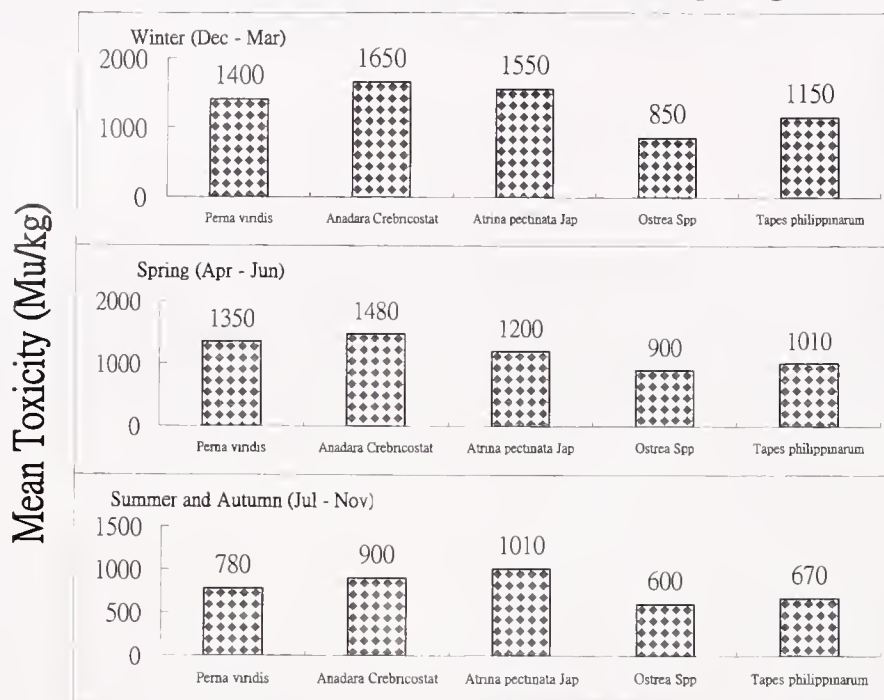


Figure 1. Mean PSP toxicity levels in various species of bivalves collected from markets of Hong Kong and the eastern South China coast.

of the total PSP toxins. GTX₁₋₄ were also high in weight percentage, but CTXs comprised only a small amount (Figs. 3 and 4).

By comparing the weight percentages of STX, GTX, and CTX toxins in shellfish samples, it was discovered that shellfish originated from different locations exhibited strong characteristics of grouping and similarities. The toxin species in green-lipped mussels originating from Macau, Hong Kong, and Mirs (Daipeng) Bay exhibited the highest similarity in weight percentages. The PSP toxin contents in green-lipped mussel originating from Daya Bay were quite different from those of the other originating areas.

TABLE 1.

Toxic phytoplanktons identified in Hong Kong and the eastern coast of the South China Sea.

Area	Species	Occurrence	Season
Southeastern Hong Kong waters	<i>Alexandrium catenella</i>	^a	Feb.-April
Mirs (Daipeng) Bay	<i>Alexandrium tamarensis</i>	^b	Jan.-April
Mirs (Daipeng) Bay	<i>Challanella marina</i>	^c	March-May
Eastern Hong Kong, Mirs Bay, and Daya Bay	<i>Gonyaulax polyedra</i>	^b	March-June
Southern and Eastern Hong Kong waters	<i>Gymnodinium breves</i>	^b	March-May

^a Often, normally > 250 cell/mL.

^b Occasionally, normally > 100 cell/mL.

^c Rare, normally less 10 cell/mL.

For bloody cockle samples, there was a very high similarity between the samples originated from Hong Kong and Macau. However, those samples originating from Mirs (Daipeng) Bay and Daya Bay exhibited another group of similarity. On the basis of the similarity in weight-composition of PSP toxins in green-lipped mussel and bloody cockle, it is able to group the PSP toxicity in two major categories; that is, the "Pearl River Estuary" group of shellfish toxins and the "South China Sea Oceanic" group of shellfish toxins. The first group includes toxins of indigenous shellfish in Macau and Hong Kong. The later group includes the PSP toxins of shellfishes cultivated in the eastern coast of South China Sea (namely, Daya Bay and Mirs Bay). Interestingly, the toxin contents for green-lipped mussel and bloody cockle that were marketed in Hong Kong exhibited high similarity to those originating from Daya Bay (where is believed to be a major shellfish cultivation water in the South China coast). The results suggested that most of the PSP-contaminated shellfish marketed in Hong Kong might be imported from the eastern coast of South China Sea in the mainland of China.

DISCUSSION

Reports on PSP intoxication are rare in Hong Kong and China. However, the impacts might be much higher than reported, because, in the past, there was insufficient awareness of the disease, which resulted in a general lack of statistical data. Before 1989, neither PSP nor toxic algal bloom were recorded in Hong Kong and China. In 1989, a bloom of the harmful phytoplankton; namely, *Alexandrium catenella*, was reported in the eastern waters of Hong Kong (Ho and Hodgkiss 1993). This harmful algal bloom resulted with a peak of 13,000 μ /kg (in average >4,000 μ /kg) of PSP toxins being detected in the cultivated shellfish. The Department of Health, therefore, banned the sale of all shellfish for 2

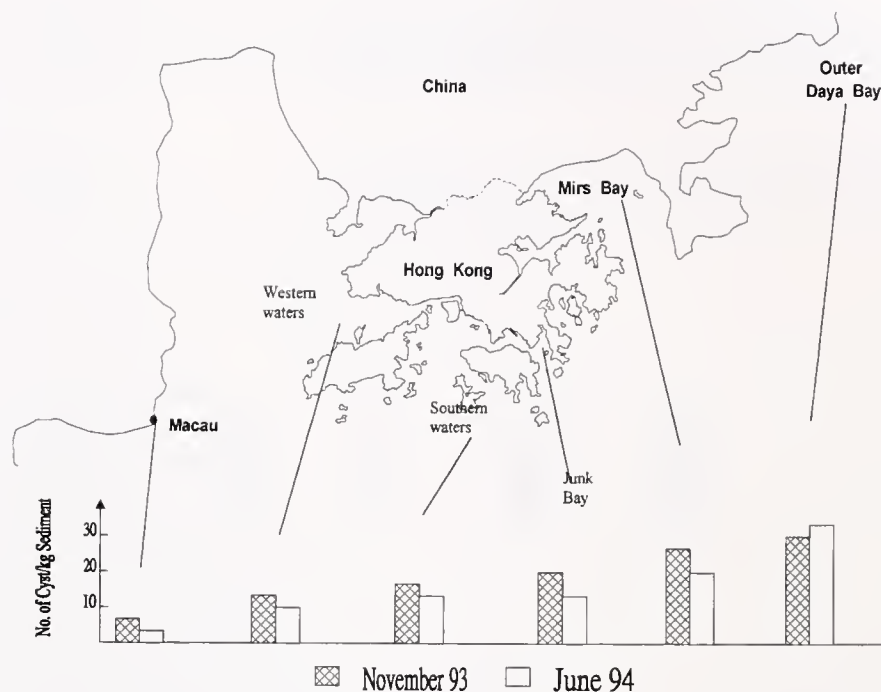


Figure 2. Distribution of *Alexandrium* cysts in Hong Kong and the eastern coast of the South China Sea.

weeks to safeguard the health of citizens. In this event, no person was affected or hospitalized.

The bloom of *A. catenella* re-occurred in the early spring (Feb.–March) of 1990, again in the eastern waters of Hong Kong. Because of the early warnings given by the Hong Kong Government, the shellfish market was not closed, but no person became ill (Ho and Hodgkiss 1993).

The most severe PSP event in China occurred in 1992 (Lin et al. 1994). In that event, one person died, and 17 persons were hospitalized. The massive intoxication happened in the eastern

coast of the South China Sea about 200 km from Hong Kong. Although no phytoplankton data were collected in this event, *A. tamarense* was suspected to be the causative organism, because this species occurred frequently in the eastern coast of South China Sea (Qi et al. 1995). The various PSP events in the late eighties and early nineties suggested that *Alexandrium catenella* and *A. tamarense* were the major causative organisms of PSP in Hong Kong and its vicinities.

The waters of Hong Kong and the eastern coast of the South China were reported to have been eutrophicated since the mid-

Composition of PSP Toxins in Green-lipped mussel (*Perna viridis*)

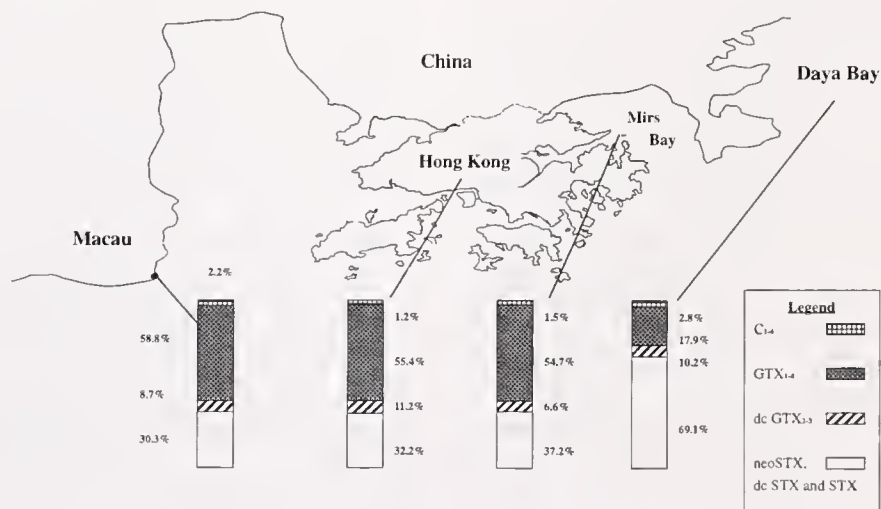


Figure 3. Weight-composition of PSP toxins in green-lipped mussel originating from Hong Kong and the eastern coast of the South China Sea.

Composition of PSP Toxins in Bloody Cockle
(*Anadara crebricostata*)

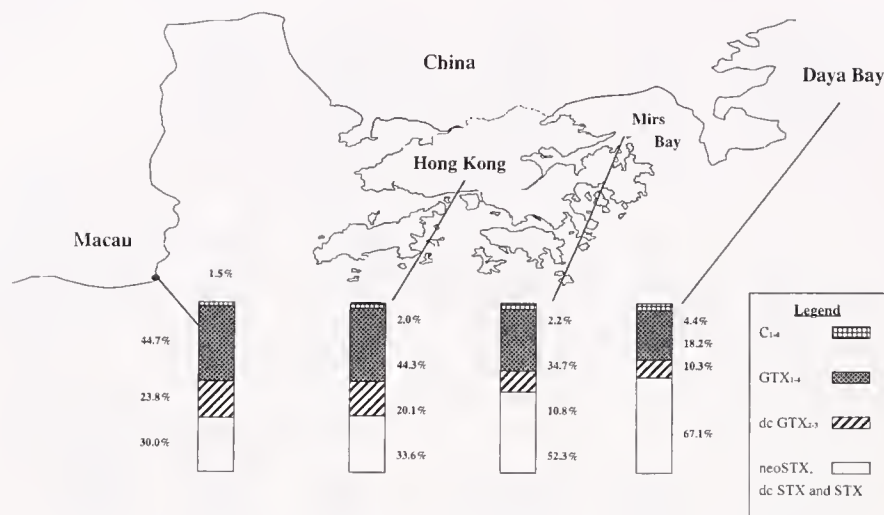


Figure 4. Weight-composition of PSP toxins in bloody cockle originating from Hong Kong and the eastern coast of the South China Sea.

eighties (Environmental Protection Department 1996). However, the phenomenon of eutrophication is not correlated with the increase in PSP intoxication and level of PSP toxins in shellfish samples of the areas. In Hong Kong, for example, the number of red tides in Tolo Harbour was highest in 1989 but gradually decreased to one-third of its highest magnitude in the midnineties. No evidence has shown that eutrophication resulted in higher PSP contents in shellfish samples of Tolo Harbour. Better pollution control in recent years did not result in a decrease of PSP contents, according to the present monitoring data.

Nevertheless, on the basis of the information of algal cyst distribution and the hydrographic conditions in Pearl River Estuary, Hong Kong, and the South China coast, it can be concluded that the intruded oceanic currents from the South China Sea during

winter might be the major carrier of harmful phytoplanktons. Qi et al (1995) and Ho and Hodgkiss (1995) reported that when the freshwater of Pearl River and the oceanic currents met at the eastern waters of Hong Kong, the upwellings provided nutritious conditions for germination and growth of toxic algae. The similarity between seasonal patterns of algal blooms and the PSP levels in the eastern coast of South China also supports the hypothesis that PSP is closely related to algal blooms that were initiated by the oceanic currents intruded into the region during late winter to spring.

As noted, some of the PSP-contaminated shellfish sold in the Hong Kong market were imported from the eastern coast of the South China Sea. Further research and management work must be done to tackle the risk of cross-border contamination.

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POSSIBLE RELATION BETWEEN A WINTER EPIDEMIC OF ACUTE GASTROENTERITIS IN FRANCE AND VIRAL CONTAMINATION OF SHELLFISH

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ABSTRACT Several outbreaks of gastroenteritis related to the consumption of shellfish (frequently eaten raw) have been reported in different parts of the world. In Europe, human calicivirus infections may have been involved in winter outbreaks in recent years, although there is little evidence confirming such viral contamination in shellfish. This study presents the first results of a field survey on viral contamination in two shellfish harvesting areas along the French Mediterranean coast. The first, consisting mainly of oyster beds, was classified in category A, as determined by fecal coliform counts in shellfish (European Community Directive 91/492), and the second, a mussel bed, was classified in category C. Shellfish samples were collected monthly between August, 1995 and April, 1997, and RT-PCR was used to detect viruses known to be involved in outbreaks of gastroenteritis: enterovirus, human calicivirus, rotavirus, and astrovirus. Contamination by fecal coliforms was evaluated in the same samples. Virological results in shellfish were correlated with data on the incidence of epidemics of gastroenteritis in the coastal population obtained from a French survey. A relationship was observed between virological results and epidemiological data. For the 2 years when the incidence rate of gastroenteritis was maximal in winter, the mussel bed was always contaminated by the four types of viruses screened. Similar results were observed for oyster beds during the second winter; whereas, two samples were highly contaminated during the first winter, and a third showed low contamination (only rotavirus). These results suggest that an epidemic of gastroenteritis in the human population contributed to viral contamination of the marine environment through discharge of waste water.

KEY WORDS: shellfish, viral contamination, enterovirus, human calicivirus, rotavirus, astrovirus, fecal coliforms, gastroenteritis

INTRODUCTION

Enteric viruses are dangerous pathogens able to persist in the environment. They are introduced into marine and estuarine waters because current sewage-treatment practices are inadequate to eliminate them from wastewater effluents. Once in the environment, they can be accumulated by shellfish and lead to outbreaks among human consumers. Shellfish-borne diseases include hepatitis A, the most serious infection, which can prove fatal, and gastroenteritis, probably the most frequent pathology. Consequently, viral contamination of shellfish and shellfish farming areas has become a matter of increasing public health concern (Gerba and Goyal 1978, Metcalf et al. 1995).

In the 1980s, cell culture methods were used to detect enteroviruses (EV) in the tissues of clams and oysters in the United States (Vaughn et al. 1980, Wait et al. 1983). However, until recently, viral pathogens have rarely been identified in shellfish-associated outbreaks because of the lack of a sensitive method for detecting viruses not amplified in cell cultures. New analytical methods based on molecular biology now allow the detection of such enteric viruses as hepatitis A virus (HAV), human calicivirus (Hu CV), and rotavirus (RV) in environmental samples and shellfish implicated in food-borne outbreaks (Le Guyader et al. 1994, 1996a, Lees et al. 1995, Chung et al. 1996, Häfliger et al. 1997).

Some viruses detected in the coastal environment have been associated with gastroenteritis. Diarrhea is commonly reported among associated symptoms in infections by a number of enteroviruses (Melnick 1996). Hu CV, including Norwalk virus, have been responsible for gastroenteritis in children and adults, astroviruses (AV) are common causes of diarrhea in children, and RVs are the most important etiologic agents of severe diarrheal illness in in-

fants and young children (Kapikian 1996). This study investigated the occurrence of these viruses in shellfish collected over a 21-month period from Mediterranean beds. Virological results were correlated with data for the incidence of epidemics of gastroenteritis among the coastal French population and are considered in terms of their implications for public health.

MATERIALS AND METHODS

Environmental Sampling

Shellfish samples were collected monthly between August 1995 and April 1997 in two harvesting areas along the French Mediterranean coast. Site 1 was an oyster (*Crassostrea gigas*—Thunberg, 1793) bed producing 10,000 tons per year. Oysters were collected from three sampling points of this shellfish area classified as category A on the basis of fecal coliform counts (European Community Directive 91/492). Site 2, about 30 km from site 1, had a low mussel (*Mytilus galloprovincialis*—Lamarck, 1819) production of 50 tons per year. Mussels from this category C shellfish area were collected at a single sampling point.

Microbiological Analysis

Quantitative estimations of fecal indicators (*Escherichia coli*) in shellfish were performed by conductance measurement (Dupont et al. 1996). For viral analysis, shellfish were shucked and dissected. Viruses were eluted from digestive tissues and concentrated by precipitation. After proteinase K treatment, nucleic acids were purified and RT-PCR performed (Atmar et al. 1995). Amplification products were detected by electrophoresis and confirmed with specific probes. For EV, the region amplified by RT-

PCR corresponded to the 5' untranslated region; whereas, for Hu CV, it was the region coding for polymerase, for AV the 3' untranslated region, and for RV a portion of protein VP7 (Le Guyader et al. 1994, 1996b, Mitchell et al. 1995).

Data Analysis

Shellfish viral contamination was evaluated monthly at each sampling point according to an index of the frequency of gastroenteritis viruses calculated as follows:

$$I = [(V_{EV} + V_{CV} + V_{RV} + V_{AV})/n] \times 100$$

where V for each virus (EV, Hu CV, RV, or AV) is expressed as positive (value of 1) or negative (value of 0); and n is the number of viruses analyzed in shellfish (n = 4).

Acute gastroenteritis in the French population was estimated using the French Sentinel System for Monitoring of Communicable Diseases (Flahault et al. 1995). Data on morbidity for seven communicable diseases were collected on a continuous basis via Videotex terminals from about 500 general practitioners. Results are expressed as the incidence rate per 100,000 inhabitants for acute diarrhea. These data were available on a local scale for this study.

RESULTS

The seasonal results for each virus detected among 83 samples analyzed are shown in Figure 1. Site 2 (category C) was contaminated much more heavily and frequently than site 1 (category A). However, the latter was not virus-free, and contamination was greater in autumn and winter, particularly in 1996. In site 1, RV were present in all samples collected during the winter of 1995, and Hu CV were detected simultaneously at all sampling points in the autumn of 1996. In site 2, Hu CV were only present in autumn and winter during both years.

Figure 2 shows the monthly occurrence of viral contamination from August 1995 to April 1997 based on the index of viral contamination expressed as the arithmetic mean of all sampling points (site 1 + site 2). Seasonal variations in pollution are apparent, with increases in autumn and a maximum in December and/or January, depending upon the year. Changes in bacterial contamination, expressed as the geometric mean of *E. coli* for all sampling points, show no apparent relationship between virus occurrence and coliform bacteria counts in shellfish. Similarly, bacterial contami-

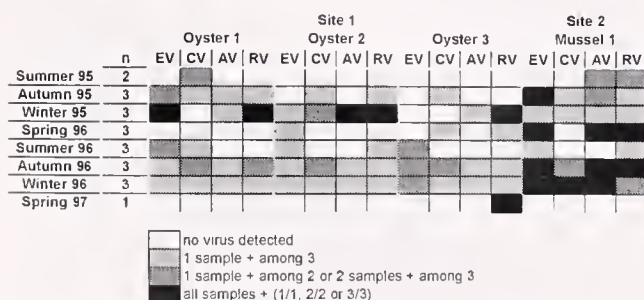


Figure 1. Viral contamination of shellfish between August 1995 and April 1997; Summer = July, August, September; Autumn = October, November, December; Winter = January, February, March; Spring = April, May, June; n = number of sampling months; EV, enterovirus; CV, human calicivirus; AV, astrovirus; RV, rotavirus.

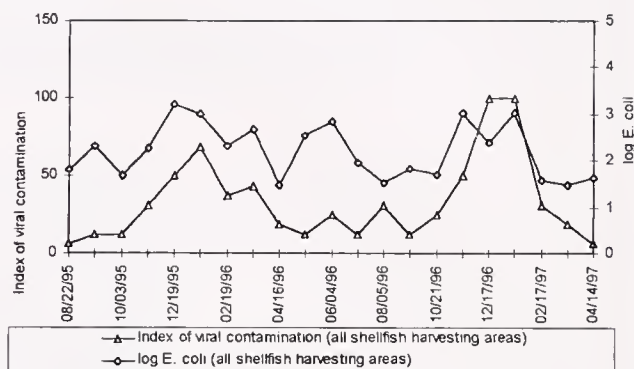


Figure 2. Comparison between index of viral contamination and shellfish bacterial contamination (all shellfish harvesting areas).

nation is apparently not correlated with the winter epidemic of acute gastroenteritis expressed as the local incidence rate per 100,000 inhabitants for acute diarrhea (Fig. 3). However, shellfish viral contamination seems to be correlated with the winter epidemic of acute gastroenteritis, with both indicators showing an increase in autumn and a peak in January (Fig. 4). This relationship was also observed for each site, as indicated for site 1 in Figure 5. Viral contamination was maximal in January, 1995, persisted until March, 1996, and was then apparent in December, 1996 and January, 1997. Results for site 2 did not show a clear relationship, probably because sampling was performed at a single point.

DISCUSSION

These results indicate that enteric viruses in shellfish were detected mainly in autumn and winter in the two sampling areas. No clear relationship was found between occurrences of viruses and coliform bacteria counts in shellfish. The variations in viral contamination seemed to follow the pattern of the winter epidemic of acute gastroenteritis in the French coastal population. These results suggest that the human epidemic contributed to viral contamination of the marine environment. However, our investigation, based on few data, provide only descriptive results and do not demonstrate a causal relationship. Further studies and more data are required to test the hypothesis statistically.

In France, large outbreaks of acute gastroenteritis occur every year during 4 or 6 weeks (end of December to January) (Flahault et al. 1997). This winter epidemic is caused mainly by a recent contact with someone with diarrhea but not by shellfish or tap

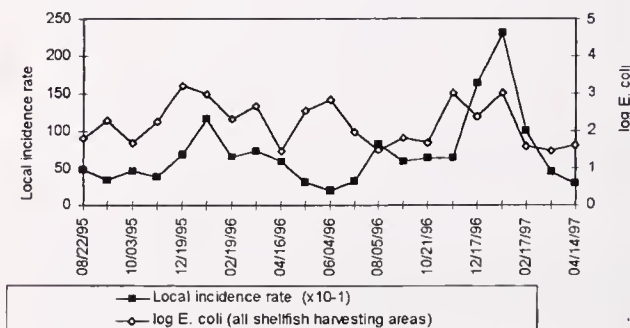


Figure 3. Comparison between winter epidemic of acute gastroenteritis and shellfish bacterial contamination (all shellfish harvesting areas).

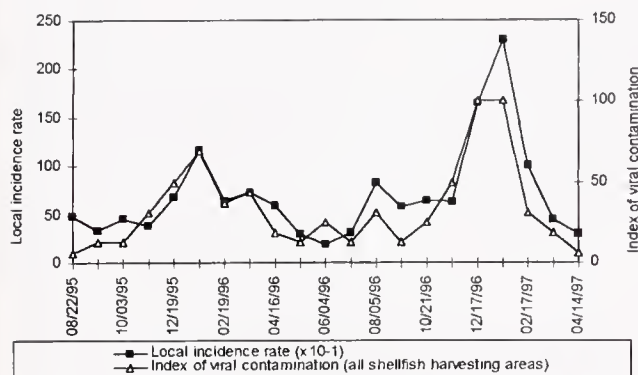


Figure 4. Comparison between winter epidemic of acute gastroenteritis and shellfish viral contamination (all shellfish harvesting areas).

water consumption (Letrilliart et al. 1997). Microbiological analyses have been unfrequently performed in stools, and few data are available on viral etiologic agents of diarrheal diseases (Maison et al. 1997). The proportion of each specific virus involved in these gastroenteritis epidemics remains unknown, but rotaviruses and caliciviruses are the most often reported etiologic agents. These viruses could be found in wastewater effluents (data not shown). Then, through sewage discharge, they could contaminate the marine environment (Dubois et al. 1997). The gradual changes of the enteric viruses in this environment are still unknown, and this question raises the problem of health hazards associated with shellfish consumption.

However, few data have been published in France on outbreaks of gastroenteritis related to shellfish consumption in winter. They occurred sporadically and locally. For example, one epidemiological study has demonstrated that an outbreak in December 1992 was statistically related to shellfish consumption. This epidemic, caused by a calicivirus, occurred in an area close to site 1, where the population was accustomed to eating local shellfish, which were the suspected vector for infection (Daurat 1992).

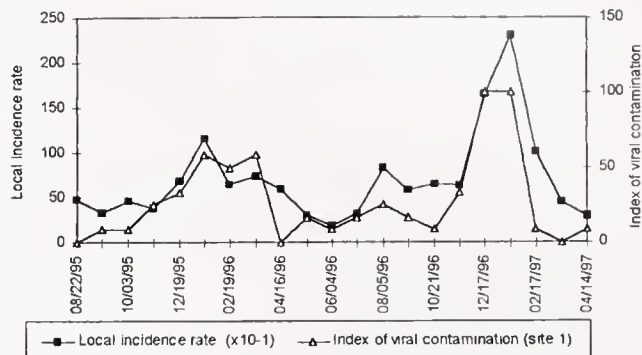


Figure 5. Comparison between winter epidemic of acute gastroenteritis and shellfish viral contamination (site 1).

Specific actions should prevent viral contamination of shellfish and reduce the public health hazards:

- (1) an efficient epidemic survey in the coastal population linked with a viral survey of sewage outputs in coastal environment;
- (2) the improvement of sewage treatment and, thus, of coastal water quality;
- (3) the respect of shellfish harvesting area classification in accordance with the European Community Directive. However, this regulation is based on the use of fecal coliform counts to assess the safety of shellfish beds, and this indicator is not an accurate reflection of shellfish viral contamination. Alternative indicators for viral pollution must be investigated and estimated according to enteric virus occurrence.
- (4) Shellfish depuration: more research is needed to improve depuration efficiency for removing viruses.

ACKNOWLEDGMENT

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INCIDENCE AND DETECTION OF PATHOGENIC *VIBRIO* SP. IN A NORTHERN NEW ENGLAND ESTUARY, USA

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ABSTRACT Many *Vibrio* species are capable of causing infections in humans. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are part of the normal microflora of estuaries and have been implicated in diseases from consumption of raw or undercooked shellfish. In the Great Bay Estuary of Maine and New Hampshire, oysters (*Crassostrea virginica*) and soft-shell clams (*Mya arenaria*) are harvested for commercial and recreational purposes. Only one incidence of *V. parahaemolyticus* infection from shellfish consumption has been documented. Traditional methods and a gene probe assay were used to enumerate *V. vulnificus* in water from sites along salinity gradients from two tributaries to the main water body (Great Bay) of the estuary. *V. parahaemolyticus*, *Escherichia coli*, enterococci, fecal coliforms, nitrate, ammonium, orthophosphate, suspended solids, chlorophyll *a*, dissolved organic carbon (DOC), temperature, and salinity were also measured. Results showed lower salinity and higher concentrations of dissolved nutrients, suspended solids, fecal indicator bacteria, and chlorophyll *a* in tributaries compared to Great Bay. Both *Vibrio* sp. were detected more frequently and at higher concentrations in the tributaries. Multiple regression analysis suggested suspended solids were the most significant variable, accounting for ~27% of the variance in *V. vulnificus* and *V. parahaemolyticus* concentrations. However, the gene probe results showed DOC was the most significant variable for explaining (44%) the variance in *V. vulnificus* concentrations. The results suggest that improved detection methods can enhance the understanding of environmental conditions conducive to both growth and inhibition of these pathogens.

KEY WORDS: *Vibrio vulnificus*, *Vibrio parahaemolyticus*, gene probe, nonpoint source pollution

INTRODUCTION

Shellfish consumed raw or partially cooked may contain pathogenic microorganisms capable of causing diseases in humans. Many of the historical problems have been linked to fecal contamination of harvesting waters, where pathogens associated with untreated sewage discharged into coastal waters are taken up and retained by filter-feeding bivalve mollusks. In response, federal and state agencies have worked to eliminate fecal contamination sources and have instituted monitoring programs with strict standards to restrict harvesting to safe waters only. This strategy has been relatively effective in limiting shellfish-borne illnesses in the United States.

Fecal-borne pathogens are not the only causes of shellfish-borne diseases. There are also many autochthonous estuarine and marine bacteria that are pathogens. In particular, pathogenic *Vibrio* spp. pose the greatest public health hazard in the United States from among all naturally occurring bacterial pathogens associated with shellfish (Janda et al. 1988, Rippey 1994). *Vibrio vulnificus* is the most serious public health concern, but *Vibrio parahaemolyticus* and *Vibrio cholerae* are bacteria that can also cause gastroenteritis symptoms that may be more severe than sewage-borne viral and bacterial pathogens (Rippey 1994). Vibrios are prevalent in warm estuarine areas throughout the world. In the United States, virtually all *V. vulnificus* infections occur from consumption of oysters harvested from the Gulf of Mexico (Watkins 1994, Rippey 1994). However, *V. vulnificus*, as well as *V. parahaemolyticus*, have been detected throughout all U.S. coastal areas (Wright et al. 1996, Oliver et al. 1983) as far north as Maine on the Atlantic coast (O'Neill et al. 1990, Bartley and Slanetz 1971) and Washington State on the Pacific coast (Kelly and Dan Stroh 1988).

In the Great Bay Estuary of Maine and New Hampshire, *V. vulnificus* was first detected in 1989 (O'Neill et al. 1990), while the presence of *V. parahaemolyticus* has been reported since 1971 (Bartley and Slanetz 1971). O'Neill et al. (1992) showed that *V.*

vulnificus was only present in the estuary from late June to early October and was more prevalent in the tidal tributaries compared to more saline, larger water bodies. Bartley and Slanetz (1971) also observed decreases in concentrations of *V. parahaemolyticus* in November, with the onset of colder water temperatures. Other studies suggest that the optimum temperature and salinity for *V. parahaemolyticus* are 20°C and 20 ppt, respectively (Grimes 1991, Rivera et al. 1989, Kelly and Dan Stroh 1988, Watkins and Cabelli 1985). The usefulness of temperature and salinity for predicting densities of *V. vulnificus* in different coastal regions of the United States has been reported (Tamplin 1994). Thus, the presence of both vibrios is affected to a great extent by salinity and temperature.

The lower salinity tributaries of the Great Bay Estuary are also known to be enriched in nutrients, suspended solids, and phytoplankton (Langan and Jones 1996). It is possible that the survival of *V. vulnificus*, and possibly *V. parahaemolyticus*, may be enhanced in nutrient-enriched areas of the estuary. Previous studies suggest that the growth of autochthonous pathogens, including vibrios, may be enhanced in coastal waters receiving nutrients from wastewater and surface runoff (Watkins and Cabelli 1985, Grimes et al. 1985). In this study, spatially intensive sampling was undertaken over a 3-year period to determine environmental variables that may help predict the incidence and concentrations of the target vibrio species, based on a larger study (Jones et al. 1997). In addition, a gene probe detection method (Summer-Brason 1998) was used to aid in the detection of the *V. vulnificus*.

MATERIALS AND METHODS

Field Sampling Sites

The concentrations and occurrence of target bacteria, dissolved nutrients, and other environmental variables were measured along transects starting from the tidal dams in the Squamscott and Oyster

ivers and ending at Adams Point between Great and Little Bays (Fig. 1). Water samples were collected mainly from seven sites differentially affected by pollution at least bimonthly beginning in June, 1993 to November, 1995, except in months where the bay was frozen.

Sample Collection

Water samples were collected at low tide. Bacteriological samples were collected in sterile 1-L polycarbonate bottles from approximately 10 cm below the surface. Water samples were kept on ice in coolers until processed at the lab no later than 20 hours from collection time. A multipurpose meter (YSI Model #33, YSI Incorporated, Yellow Springs, OH) was used to determine temperature and salinity *in situ*. An oxygen meter (YSI Model #51B, YSI Incorporated) was used to measure dissolved oxygen.

Bacteriological Isolation and Enumeration

Water samples were inoculated into a multiple tube fermentation (MPN) analysis series with an alkaline peptone water (pH 8.6, 1% NaCl) selective enrichment. The MPN tubes were incubated at 35°C for 18 to 24 h, and turbid tubes were streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) and Colistin-Polymixin B-Cellobiose (CPC) (Oliver et al. 1992) agar plates. Both TCBS and CPC plates were incubated at 36°C for 18 to 24 h. *Vibrio* isolates were streaked for purity onto brain heart infusion agar supplemented with 1% NaCl. Isolated pure colonies were inoculated into salt tolerance and differential media tests. Isolates were classified as presumptive *V. vulnificus* or *V. parahaemolyticus* based on their growth in peptone salt broths, reactions in arginine dehydrogenase, lysine decarboxylase, and ornithine decarboxylase media, and fermentation of cellobiose and salicin (Jones et al. 1991). Presump-



Figure 1. Sampling sites in the Great Bay Estuary. Inset: The location of the Great Bay Estuary between Maine, New Hampshire, and the Atlantic Ocean.

tive isolates were frozen at -70°C until further confirmation using the API 20E identification system (Analytab Products, Inc., Plaineville, NY) for both vibrios and by the polymerase chain reaction (PCR) for *V. vulnificus*. PCR reactions were carried out according to the protocol of Hill et al. (1991).

Water samples were also examined for enterococci, *Escherichia coli*, and fecal coliforms. Samples were through sterile 0.45 μm -pore size Gelman filters. Filters were placed on mE agar (Difco) for enterococci enumeration and mTEC media (Difco) for fecal coliform and *E. coli* enumeration and incubated for 48 h at 41°C and 18 to 24 h at 44.5°C , respectively (U.S.E.P.A. 1986). All yellow colonies on mTEC agar were counted as fecal coliforms; *E. coli* colonies were enumerated by transferring the filters onto cellulose pads soaked with urea and counting the remaining yellow colonies.

Isolation and Identification of *V. vulnificus* with an Oligonucleotide Gene Probe

Each of the samples examined by traditional culturing techniques during 1995 were also cultured onto Luria agar supplemented with 1% NaCl. Plates were incubated at room temperature for 48 to 72 h. Plates containing approximately 100 colonies were used to make colony blots. Colony blots were made according to the procedures outlined by Wright et al. (1993), with a few modifications. Prepared filters were air dried and stored in a plastic bag until hybridization.

The *V. vulnificus* fluorescein labeled gene probe was based on a portion of the *V. vulnificus* cytolysin structural gene, consisting of the following oligonucleotide sequence: GAGCTGTCACG-GCAGTTGGAACCA, which has 100% specificity and sensitivity for *V. vulnificus* (Wright et al. 1993). A fluorescein label (National Biosciences Inc., Plymouth, MN) was used instead of the alkaline phosphatase label on the 5' amino terminal of the oligonucleotide. Hybridization procedures and solution preparations were taken from Genius System User's Guide for Membrane Hybridization Version 3.0 (Boehringer Mannheim Corp., Indianapolis, IN), with some modifications in times and wash orders. Hybridization of the probe was detected colorimetrically using an antifluorescein alkaline phosphatase antibody and the addition of detection reagents. The alkaline phosphatase activity detection was assayed colorimetrically with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate tablets dissolved in distilled Milli-Q water. Control strains of *V. vulnificus*, *V. parahaemolyticus*, *V. fluvialis*, and *V. cholerae* were run with each batch of filters.

Nonmicrobiological Water Analysis

Acid-washed 1-L polyethylene bottles were used for additional water analysis. pH was measured using a Fisher Accumet pH meter. Samples were shaken and 500 mL filtered through predried and preweighed glass fiber filters (1.2 μm nominal pore retention). The filters were rinsed with deionized water to remove salts and dried for 24 h at 80°C . Filters were weighed to determine weight of suspended solids. These filters were then combusted at 450°C and reweighed to yield the percent organic content of the filtered solids. The other 500 mL of the water sample was also filtered through a glass fiber filter with 0.2 mL of MgCO_3 solution added to the sample after approximately 475 mL had been filtered. The filtrate was separated into three acid-washed polyethylene bottles (50 mL size) and used for nutrient concentration assessment. The

samples were analyzed in duplicate for nitrate, nitrite, phosphate, and ammonium using a LACHAT "QuikChem" method. The filter was analyzed for chlorophyll *a* and phaeopigments either immediately or the filter was frozen at -80°C and analyzed within 7 days. DOC was measured with a Shimadzu TOC 5000 (platinum-catalyzed high-temperature combustion) (Sugimura and Suzuki 1988).

Statistical Analysis

Bacterial enumeration data were adjusted with a log transformation to normalize the distribution of the data. Statistical analysis of traditional culturing techniques and the oligonucleotide probe were performed using the chi-square test and Excel software. The effects of all environmental variables (DOC, DON, nitrate, ammonium, orthophosphate, chlorophyll *a*, dissolved oxygen, pH, temperature, salinity, total suspended solids, total organic suspended solids) on concentrations of a bacterial species were examined using step-wise forward multiple regression models and JMP software. Differences were considered significant at $p < .05$ for all analysis.

RESULTS

Water samples were collected at low tide at the seven sites from July, 1993 to November, 1995. For this study, only data collected during the warmer months of May through November were used. Temperature and pH measurements did not show much variation between sites. However, most other parameters showed gradients from Great and Little Bays up into the tributaries. The over-all average salinity was higher in the bays (GB1, GB2, OR1) and decreased going up into the Oyster (OR6 and OR10) and Squamscott (GB4 and GB7) rivers (Fig. 2). Most of the nutrient and microbial concentrations increased along the two gradients from the bays into the rivers, as illustrated for overall average fecal coliform concentrations in Figure 3.

Both *V. vulnificus* and *V. parahaemolyticus* were detected only from May until November. Figure 4 shows the responses of both bacteria to increasing temperatures at all study sites. Relatively low concentrations were first detected in late May (1995 only), with sharp increases from June to July and August with increasing temperatures. Bacterial concentrations declined in September and October before disappearing again in November. Other studies have shown these species to become nondetectable in water where temperatures drop below 10°C (Wright et al. 1996, O'Neill et al. 1992, Bartley and Slanetz 1971).

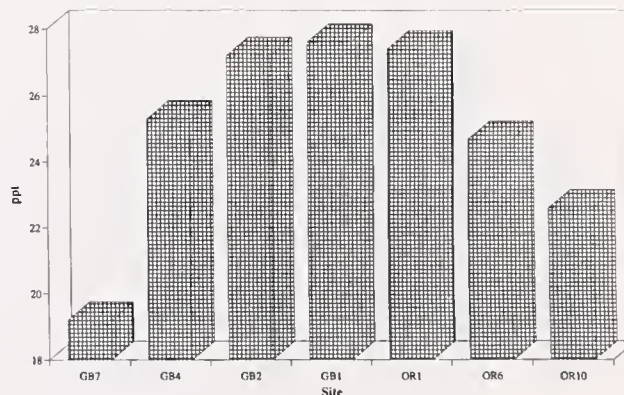


Figure 2. Average salinity (ppt) during low tide at sampling sites: 1993 to 1995.

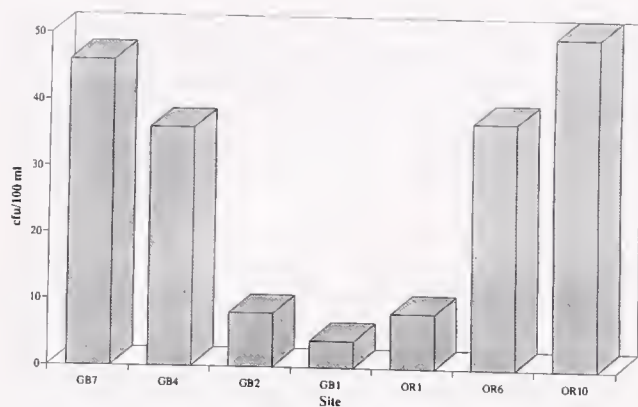


Figure 3. Average fecal coliform concentrations at sampling sites: 1993 to 1995.

By using only data from sample dates where the vibrios were detected, an in depth assessment of the effects of water quality variables on vibrio concentrations was undertaken. Overall average vibrio concentrations at the seven sampling sites are illustrated in Figure 5. The same general gradient in concentrations relative to location along the sampling transects as seen for other water quality variables was apparent for the two vibrios. Multiple stepwise regression analysis was used to determine whether the water quality parameters could be used to predict vibrio concentrations. Despite some differences between years, total suspended solids (TSS) was always a significant variable for *V. vulnificus* concentrations, and was also a significant variable for *V. parahaemolyticus* in two of the three years. The regression analysis based on the whole 1993–1995 data showed TSS accounted for 29% of the variation in *V. vulnificus* concentrations, with temperature and salinity as two significant subordinate variables. TSS accounted for 26% of the variation in *V. parahaemolyticus* concentrations with temperature, orthophosphate and total organic suspended solids as significant secondary variables.

The gene probe assay was used as an attempt to provide more consistent detection of *V. vulnificus* compared to the traditional method of detection. The gene probe assay allowed the detection of *V. vulnificus* more frequently and at higher concentrations (Fig. 6). Multiple stepwise regression analysis identified dissolved organic carbon (DOC) as the variable accounting for the majority (44%) of the variation in *V. vulnificus* concentrations, and TSS was not a significant variable.

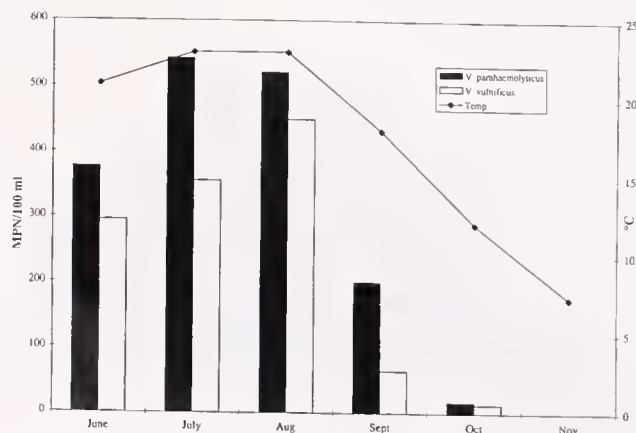


Figure 4. The average monthly water temperature and concentrations of *V. parahaemolyticus* and *V. vulnificus* at all sampling sites: 1993 to 1995.

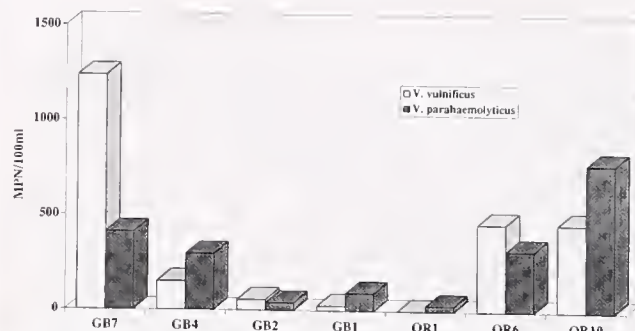


Figure 5. The average concentrations of *V. parahaemolyticus* and *V. vulnificus* at sampling sites: 1993 to 1995.

DISCUSSION

The 3 years of data for *V. vulnificus* and *V. parahaemolyticus* indicated spatial and temporal variability for these bacteria in the Great Bay Estuary. These results suggest that the two species may occupy particular niches that shift in response to changes in environmental conditions. Despite specific differences, there were general spatial and temporal similarities in incidence and concentrations of the vibrios. Both species were present only during warm months and tended to be present in higher concentrations in the tributaries. Previous studies of O'Neill et al. (1992) had first described the variability in distribution of *V. vulnificus* in the Estuary, and Bartley and Slanetz (1971) had earlier described similar observations for *V. parahaemolyticus*. Rivera et al. (1989) found differences in the distribution of *V. vulnificus* and *V. parahaemolyticus* in tropical waters where both species occurred.

The effects of environmental conditions on the incidence of *V. vulnificus* and *V. parahaemolyticus* was determined using measurements of many different water quality variables made at the same time as sampling for vibrios was conducted. The results indicated that TSS was a significant factor for explaining the variance in vibrio concentrations. Others have shown strong relationships between *V. parahaemolyticus* and plankton concentrations, especially copepods (Venkateswaran et al. 1989, Kaneko and Colwell 1978). Watkins and Cabelli (1985) also suggested a relationship between *V. parahaemolyticus* and elevated concentrations of plankton that respond to nutrient pollution associated with sewage

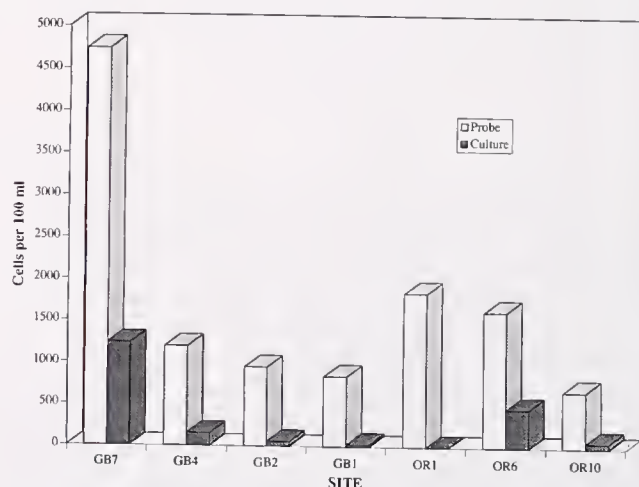


Figure 6. The average concentrations of *V. vulnificus* detected at sampling sites by gene probe (Probe) and traditional culture (Culture) methods: 1993 to 1995.

discharges. In our study, TSS and chlorophyll *a* were typically highest in the nutrient-enriched tributaries. However, samples collected when TSS, were especially high often resulted in no detection of the target vibrios. Thus, the mechanism of TSS influence on vibrio concentrations in the Great Bay Estuary is unclear.

The inconsistent detection of vibrios under such environmental conditions as high TSS concentrations was a concern. A true understanding of the ecology of these organisms requires accurate measurements of their concentrations. A gene probe assay based on that developed by Wright et al. (1993) was used to measure concentrations of *V. vulnificus* in 1995. The regression analysis using gene probe data suggested DOC as the most significant factor in explaining the variability in *V. vulnificus* concentrations. The relationship was positive, suggesting that *V. vulnificus* occurs in areas with elevated DOC, such as tributaries subject to organic nutrients present either from point and nonpoint source pollution or as a result of enriched primary productivity in the presence of elevated inorganic nutrients. Previous studies suggest that the growth of vibrios may be enhanced in coastal waters receiving

nutrients from wastewater and surface runoff (Watkins and Cabelli 1985, Grimes et al. 1985).

The results suggest that improved detection methods can enhance the understanding of environmental conditions conducive to both growth and inhibition of these pathogens. Gaining a better understanding of the ecology of pathogenic vibrios can help in the prediction of potential disease incidence from shellfish consumption and in the development of postharvest processing that may eliminate these pathogens from shellfish (Jones et al. 1995).

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SHOULD MOLLUSK TOXICITY IN MEXICO BE CONSIDERED A PUBLIC HEALTH ISSUE?

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ABSTRACT As high as 4,000 µg/100 g of tissue of Paralytic Shellfish Poisoning, PSP, toxin has been recorded in one single specimen of *Pinna rugosa*, obtained from the Gulf of California, México, in the absence of an evident "red tide" and with no human poisoning cases on 1993. There is no clue about how the toxin accumulated in such an organism, nor for how long the toxin remained stored. In contrast, the contamination of other mollusks, such as oysters (*Ostrea iridescens*), mussels (*Choromytilus palliopunctatus*), and goose barnacle (*Pollicipes polinerus*), with saxitoxin values as high as 3,500 µg/100 g of pooled tissue, have provoked at least 500 individuals hospitalized and the death of at least 20 persons in recent years along the Pacific coast of México. Large blooms of toxic *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum* have been associated with such events, and in lesser extent *Alexandrium* sp., *Dinophysis acuta*, and *D. acuminata* dinoflagellates have also been detected in the water body. On the other hand, the diatoms *Nitzschia pseudodelicatissima* Hasle, *Pseudonitzschia pungens* f. *multiseries*, and *P. australis*, have been linked to large ecological losses in the Gulf of California, but as yet their toxin (domoic acid) has not been detected in local shellfish species. Other toxin sources are becoming a serious threat to public health, such as those from cyanobacteria (*Trichodesmium* sp., *Nodularia* sp., and *Microcystis* sp., *Anabaena* sp., and *Aphanizomenon* sp.), affecting the aquaculture industry.

KEY WORDS: México, PSP, monitoring, *Gymnodinium*, *Pyrodinium*, *Pseudonitzschia*

INTRODUCTION

Harmful algal blooms, HABs, are now considered an economic and environmental problem worldwide. In aquaculture, HABs are responsible for mass killings of cultured organisms by different mechanisms, which include toxicity, clogging of the gills, and/or by producing an oxygen deficit in the water body. Also, the shellfish in particular become toxic by accumulation of microalgae products that can then affect people and wild life. Fortunately, only a fraction (70 species so far) of about 5,000 microalgae species in the marine environment are considered noxious, although more than 300 can produce blooms that can discolor the water producing nontoxic "red tides."

México lacks an official permanent monitoring program to follow HAB events. The information generated on this topic by Mexican scientists has been insufficient to induce a policy that would regulate and control the collection, distribution, and sale of edible shellfish from the marine environment. At its best, the health authorities demand from the producers basic hygiene measures for handling the product to avoid contamination with fecal bacteria, but the possibility of introducing a product contaminated with marine biotoxins is high. At present only three laboratories in the whole country enjoy the certification of the Health Ministry to carry out the mouse bioassay PSP-test (AOAC) to detect the presence of marine toxins, and none possess the necessary infrastructure to identify the nature of the toxin, if found. Hence, the efforts of just a handful of research workers interested in the HAB phenomena in México are receiving inadequate attention or support. It is important to recognize that some organisms often associated with HAB in the Pacific coast of México are nontoxic. Among them, the ciliate *Mesodinium rubrum* and the dinoflagellates *Ceratium furca*, and *C. tripos* var. *poncticum*, *Gonyaulax triacantha*, *G. splendens*, *Noctiluca scintillans*, and *Scrippsiella trochoidea* are the most common (Cortés-Altamirano et al. 1996). They all seem to affect the quality of the water visibility and, at high densities, provoke fish and invertebrate kills by oxygen depletion. On the

other hand, the events associated with toxic blooms on the Pacific Coast were caused mainly by *Gymnodinium catenatum* (Mee et al. 1986) or *Pyrodinium bahamense* var. *compressum* (Ramírez-Camarena et al. 1996). Environmental contingencies associated to HABs reach nearly half of total number of environmental emergencies reported by PROFEPA, the agency for environmental protection of the Ministry of Ecology of Mexico (Fig. 1). The preferred season for toxic blooms to occur seems to be early spring (around Easter) and late autumn (close to Christmas) each year. It is interesting to note that 80% of HABs reported in México have occurred on the Pacific Coast (Table 1).

From October 1995, throughout February 1996, the Health Ministry registered 195 human poisoning cases related mainly to the consumption of oysters in Acapulco's harbor. The toxin analysis revealed levels well above the WHO's standard (129–138 µg PSP/100 g of shellfish tissue), and some control measurements were adopted to preclude the extraction and marketing of toxic shellfish (Ramírez-Camarena et al. 1996). Roughly, the total economical losses were estimated in several millions of dollars. On May 7th 1997, a new outbreak, with toxicity levels above 330 µg PSP/100 g of tissue of oyster, was observed. The timely intervention of health authorities in banning consumption and marketing of shellfish yielded only seven mild poisoning cases, showing the efficacy of such a measurement. However, serious economical losses were reported by fishers. These experiences clearly indicate the benefit of establishing a permanent monitoring program, but the problem of control, avoidance and/or reducing the impact of HAB's remains a challenge.

CONSIDERATIONS FOR A HAB MONITORING PROGRAM IN MÉXICO

Its geographical location makes Mexico the fifth richest country in biodiversity on the planet. Therefore, for a sound monitoring program, basic knowledge about the biological, chemical, and physical conditions, as well as the temporal and geographic

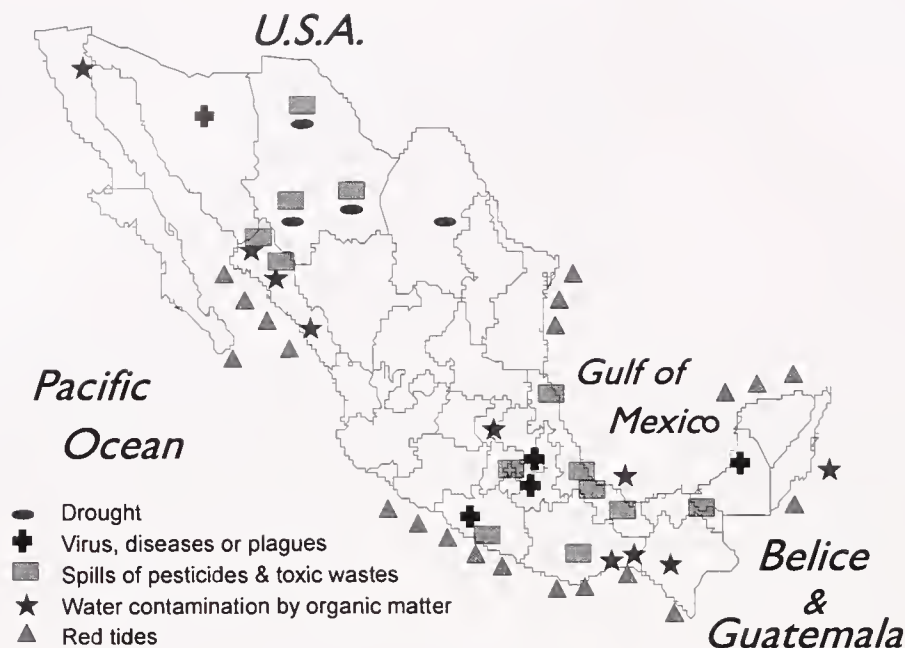


Figure 1.

variation within the region of interest must be known. With regard HAB events, the priority on information concerns the occurrence in time and space of the toxic algae, its population dynamics, and the function of the ecosystem. In this case, designing a method to determine the presence of a given dinoflagellate species is insufficient, because it is also important to understand its role in terms of both ecology and economy. It should be remembered that the potentially harmful algae exhibit genetic variability and that toxic and nontoxic strains occur within individual species. Furthermore, toxic species may show differences in potencies. Such differences seem to be genetically, as well as environmentally regulated, thus increasing the difficulty of designing a simple monitoring strategy.

The Pacific region of México lies in a transition zone that receives the influence of the temperate Alaska current from the north and the equatorial Panama current from the South. Thus, biodiversity is large and obliges us to consider monitoring of a particular species even at low densities, because the most dramatic events in which the wild population has been affected does not always occur at the peak of a "red tide," and the toxicity in shell-

fish does not correlate with such a parameter (Sierra-Beltrán et al. 1996). The dynamics of an algal bloom has three main steps: initiation, development, and decay. In the case of *G. catenatum*, upwelling seems to provoke a shift from diatom to dinoflagellate populations, leading to blooming of *G. catenatum* (Orellana-Cepeda 1993). Distribution of the phytoplankton population in a given area occurs through two mechanisms: horizontal transport of vegetative cells or germination of benthic cysts. The horizontal transportation in turn, can be produced by currents from areas where the species is commonly present to others where it is usually absent; or by such human activities as ship's ballast water or transplantation of living mollusks for aquaculture. In these cases, however, the organisms are usually in the form of resting cysts, or spores, and may explain the appearance of new species not previously reported. Thus, we should be especially careful to not underestimate the presence of very low amounts of cells that usually pass unnoticed with the current monitoring techniques available.

It has been suggested that the Central American Pacific region constitutes a system of its own, similar to other well-recognized ones, as the Indo-Pacific, Papua New Guinea, Sabah, and Philippines systems, and, therefore, deserves to be studied as a whole (Sotomayor-Navarro and Domínguez-Cuellar 1993).

The climate conditions in the Gulf of California have been changing in recent years, showing a trend to colder water temperatures. Upwelling movements are favored by strong northwest winds in the east coast of the gulf, which then produce strong southward currents. The satellite images of the last 10 years (1986 to 1996) clearly indicate an abnormally low temperature range for the region, thus confirming the above hypothesis (Sierra-Beltrán et al. 1997). Another hypothesis that may help to explain the sudden diatom blooms on the Gulf of California in recent years is related to the rise in the concentration of CO_2 (Ho and Hodgkiss 1991). Cold waters usually harbor higher concentrations of CO_2 (Au et al. 1989), and this compound limits diatom blooms (Riebsell et al. 1993). Although, and fortunately, no human casualties have been

TABLE I.
Environmental emergencies affecting the Marine Ecosystem in México, during 1996.

Cause of the Contingency	Number of Events	%	Pacific	Gulf of México	Caribbean
Biotoxins and red tides	9	45	7	1	1
Taura virus	2	10	2		
Agrochemicals	4	20	3	1	
Hydrocarbon spills (diesel)	2	10	1	1	
Human activities	3	15	1	2	
Total	20	100	14	5	1

linked to diatom blooms in México, their ecological impact has been dramatic, affecting over 766 sea birds (*Gavia immer*), and 174 marine mammals (4 *Balaenoptera physalus*, 164 *Delphinus delphis*, 3 *Tursiops truncatus*, and 9 *Zalophus californianus*). Events associated to *Pseudonitzschia* sp. blooms seem to occur mainly in temperate climates, and this year, for example, at Puerto Peñasco, Sonora, numerous marine seabirds and mammals appeared dead in the sea. The impact was even felt as far as Topolobampo, Sinaloa, some 600 miles southward of the blooming center, and the time elapsed until the last animal body was recovered was 75 days. Histopathological analysis revealed some acute lesions on liver and accumulation of Ca^{2+} in the brain, as well as vascular infiltration and evidence of some interstitial pneumonia in lung samples. All of these lesions could be linked to the effect of domoic acid, whose presence was confirmed by high-performance liquid chromatography (HPLC) analysis (concentrations up to 300–600 $\mu\text{g}/100$ g of tissue in brain, heart, and plasma). The stomach content of some of the animals analyzed also showed the presence of diatom frustules. In some of the stranded dolphins, the stomach content revealed the presence of sardine (*Sardinops sa-*

gax), which, in turn, showed accumulation of ASP in the gut content and also allowed the identification of *P. australis*.

Recorded since 1993, cyanobacteria blooms are affecting the aquaculture industry, causing severe losses on shrimp farming ponds and, also, a serious threat to public health (Cortés-Altamirano et al. 1997). *Trichodesmium* sp., *Nodularia* sp., *Microcystis* sp., *Anabaena* sp., *Anabaenopsis elenkenii*, *Anabaena flos-aquae*, *Aphanizomenon* sp., and *Oscillatoria* sp., are among the species reported causing problems in shrimp farming ponds. During winter 1996, several problems aroused on fresh water reservoirs causing fish mortalities (unpublished results). In one event, scum forming *Microcystis* sp., clogged fish gills suffocating more than 500,000 *Oreochromis mossambicus* overnight.

Another mass mortality of the same fish species was caused by an anoxic layer more than 25-m depth, entirely covering the surface of a closed natural basin. This time the culprit was a bloom of LPPB cyanobacteria. Fortunately, until now, no human poisoning event has been linked to these phenomena, but the economic effect has been severe on the rural communities that used to profit from such fish.

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THE DEVELOPMENT OF POLYMERASE CHAIN REACTION ASSAYS FOR DETECTION OF SMALL ROUND STRUCTURED AND OTHER HUMAN ENTERIC VIRUSES IN MOLLUSCAN SHELLFISH

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ABSTRACT Public health controls for molluscan shellfish are hampered by the absence of methods for the detection in shellfish of the viral pathogens causing illness. The polymerase chain reaction (PCR) offers a potential way forward; however, its application to such complex samples as shellfish is hindered by the presence of potent amplification inhibitors. We describe the development of a procedure employing a combination of virus purification and nucleic acid extraction for the removal of the majority of such PCR inhibitors from shellfish. Initial developmental work used seeded poliovirus as a model and showed that PCR sample tolerance ranged from 2 to 5 g shellfish for highly polluted samples with over-all sensitivity limits of <10 plaque-forming units (PFU) poliovirus. These methods have recently been applied to the detection of hepatitis A virus and Norwalk and related small round structured viruses (SRSVs) in shellfish. Initial seeding experiments have shown that the method for removal of amplification inhibitors is equally applicable to the detection of these viruses by PCR in shellfish. However a single round reverse transcriptase-polymerase chain reaction (RT-PCR) proved insufficiently sensitive to reliably detect SRSVs in shellfish samples associated with incidents of human infection. We describe the further development of a nested RT-PCR procedure for the detection of SRSVs in shellfish and the application of this assay for the detection of SRSV in commercially produced shellfish and in shellfish implicated in outbreaks of gastroenteritis. These studies show that detection of human enteric viruses in molluscan shellfish by PCR is feasible and may ultimately contribute to the further development of public health controls.

KEY WORDS: virus, SRSV, shellfish, nested PCR

INTRODUCTION

Gastroenteritis caused by Norwalk virus and the related small round structured viruses (SRSVs), and hepatitis A infection, is often associated with the consumption of bivalve shellfish (Murphy and Grohmann 1980, Morse et al. 1986, Pontefract et al. 1993). The inability to detect these viruses in shellfish has hampered development of diagnostic and epidemiological procedures and the application of improved control measures. Numerous methods have been proposed for the isolation and culture of human enteroviruses from shellfish (Lewis and Metcalf 1988). However, these methods are not directly applicable to detection of the difficult-to-culture hepatitis A virus or the nonculturable viruses primarily responsible for gastroenteritis. Recently, genomic RNA sequences of Norwalk virus (Jiang et al. 1993) and other SRSVs (Green et al. 1994, Green et al. 1995b, Lew et al. 1994) have become available and have led to the development of sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) assays for the diagnosis of SRSV infection (Green et al. 1995a, Moe et al. 1994). Consensus primers that detect all SRSVs have not as yet been identified, but a broadly reactive primer pair that detects approximately 90% of SRSVs circulating in the UK have been described (Green et al. 1995a). However, a major obstacle to routine application of the PCR to complex samples, such as shellfish, is the presence of amplification inhibitors. We describe the development of a procedure employing a combination of virus purification and nucleic acid extraction for the removal of the majority of such inhibitors from shellfish and the application of this technique to the direct detection of enteric viruses in field samples. The relative

similarity of the picornaviruses to Norwalk and related SRSVs suggests that the methods developed for sample concentration and PCR inhibitor removal should be equally appropriate for the detection in shellfish of these clinically important viral pathogens. We describe here the application of the developed extraction technique to the successful detection of SRSVs in shellfish. However, a single round RT-PCR proved insufficiently sensitive to reliably detect SRSVs in shellfish samples associated with incidents of human infection. We describe the further development of a nested RT-PCR procedure for the detection of SRSVs in shellfish and the application of this assay for the detection of SRSVs in commercially produced shellfish and in shellfish implicated in outbreaks of gastroenteritis.

MATERIALS AND METHODS

Shellfish

Commercially purified oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*) were used for assay development. The assay was evaluated against shellfish (*Crassostrea gigas* and *Ostrea edulis*), mussels (*Mytilus edulis*), and cockles (*Cardium edule*) subject to high levels of sewage pollution at various coastal locations in the UK. Shellfish used for enterovirus isolation and RT-PCR were stored frozen whole at -70°C prior to use.

Shellfish Processing and Viral RNA Extraction and Purification

The procedure for shellfish processing was a modification of that by Lewis and Metcalf (Lewis and Metcalf 1988) and has been

previously described in full (Less et al. 1994). Essentially, up to 50 g shellfish flesh was shucked, homogenized, sonicated, centrifuged, and supernatants precipitated using polyethylene glycol. Resuspended pellets were sonicated and centrifuged before further virus purification by extraction with 1,1,2-trichloro-2,2,2-trifluoroethane (Freon TF) followed by centrifugal concentration and storage at -20°C . Extracts at this stage were termed purified concentrates. The RNA extraction procedure has been previously described in full (Lees et al. 1994, Lees et al. 1995). Essentially, a reaction mix of glass-powder matrix and guanidine isothiocyanate (GITC) was used to extract total nucleic acid from purified shellfish concentrates. GITC served to lyse samples and protect RNA from enzymatic digestion. RNA bound to glass-powder was washed with GITC, ethanol, and acetone before elution in Tris buffer. RNA was then precipitated in ethanol and pellets stored at -70°C prior to RT-PCR.

Reverse Transcription and PCR

cDNA was synthesized from RNA pellets and the RT-PCR for enteroviruses, and the single round SRSV RT-PCR performed as previously described (Lees et al. 1994, Lees et al. 1995). The strategy for development of the nested RT-PCR has been reported elsewhere in full (Green et al. 1998). Briefly, in the first round, SRSV RT-PCR, a broadly reactive primer combination of the three primers, G1/G2/SM31, was used. The sense primers, G1 and G2 were derived from published SRSV RNA polymerase sequences and were designed to anneal specifically with Genogroup I and Genogroup II strains, respectively. The antisense primer, SM31, has previously been described (Norcott et al. 1994). The internal (nested) primers were a previously described primer pair NI/E3 that amplify a 113 base pair region of the RNA polymerase gene corresponding to nucleotides 4756-4867 of Norwalk virus (Green et al. 1995). This SRSV primer set has been shown to amplify greater than 90% of strains circulating in the UK in 1993 to 1994.

Southern Blot Hybridization of Amplification Products

Southern Blot hybridization of SRSV RT-PCR amplicons was performed to confirm specificity of amplification and to detect weakly positive samples where amplicon bands were not visible under UV radiation, as previously described (Lees et al. 1994). Agarose gels were prepared for the Southern Blot procedure by submersion for 30 min in denaturation solution followed by neutralization. Amplification products were transferred onto a positively charged nylon membrane (Boehringer Mannheim) using a standard capillary procedure and cross-linked to the membrane by baking at 120°C for 20 min. Hybridization, using a pool of four oligonucleotides, was performed at 40°C overnight. The hybridized probe was detected by chemiluminescence. Hybridization and chemiluminescent detection reagents were according to manufacturers recommendations (Boehringer Mannheim).

RESULTS

Assay Development

The application of the RT-PCR to shellfish was evaluated by extracting nucleic acid (NA) from homogenates (1:10 in TPB-glycine broth) of commercially depurated oysters (*C. gigas*) or mussels (*M. edulis*) seeded with 10^5 PFU poliovirus. RT-PCR productivity was assayed by agarose electrophoresis. Of the NA extraction procedures investigated, glass-powder matrix (Boom et

al. 1990, Yamada et al. 1990) gave the cleanest results. A modified version of the Boom (Boom et al. 1990) procedure, incorporating guanidine isothiocyanate sample lysis (and endonuclease protection) with an ethanol precipitation step for optimal elution and recovery was adopted as the NA extraction procedure. However, crude shellfish homogenates were found to contain potent RT-PCR inhibitors.

The assay was further developed by combining the NA extraction procedure with methods for the isolation of intact viruses from shellfish. RT-PCR inhibition was evaluated by seeding final extracts with 10^5 PFU poliovirus. An adsorption-elution-precipitation procedure (Sobsey et al. 1978) and a polyethylene glycol (PEG) precipitation procedure (Lewis and Metcalf 1988) were evaluated for this purpose. In our hands, the PEG precipitation procedure gave marginally more consistent virus recoveries and was adopted for further work. RT-PCR inhibition for PEG extracts of depurated shellfish was substantially reduced with RT-PCR sample tolerances in excess of 1 g for both species. However, analysis of environmentally contaminated shellfish showed that RT-PCR inhibition was still a major factor influencing sensitivity with sample tolerance of only 0.23 g for oysters and 0.8 g for mussels. RT-PCR sample tolerance is expressed as the shellfish weight, causing no more than an arbitrary 25% inhibition over that of a poliovirus control, as measured by RT-PCR band intensity on agarose gels. Final stages of assay development utilized Freon TF extraction of PEG extracts for further virus purification followed by microfugal ultrafiltration for volume reduction. RT-PCR inhibition by extracts prepared from polluted shellfish using this final form of the procedure was substantially reduced. Sample tolerances were in excess of 4 g for depurated oysters or mussels and ranged from 2 g (oysters) to 9.5 g (mussels) for polluted shellfish (Table 1). The finalized procedure for extraction, purification and amplification is shown in outline form in Figure 1.

Application to Small Round Structured Viruses

The experiments described above using an enterovirus PCR documented the potent inhibitory potential of shellfish. The application of the sample extraction procedures to the SRSVs was studied by comparing shellfish mediated amplification inhibition in both the enterovirus and SRSV PCRs. Initially, a 10% extract (in water) of acute phase human stools containing SRSV was added to control oyster extracts processed up to Freon extraction (Fig. 1, stage 5), and then nucleic acid was extracted, and RT-PCR was performed using cross-reactive SRSV primers. There was no discernible amplification inhibition when commercially depurated or

TABLE 1.
RT-PCR sample tolerance^a for contaminated^b shellfish at key stages of the extraction and purification procedure (Fig. 1).

	Oysters	Mussels
Shellfish homogenates (stage 1)	0.018	0.002 ^c
Primary extracts (stage 3)	0.23	0.8
Purified extracts (stage 5)	2	9.5

^a Expressed as the shellfish weight (in grams) causing no more than 25% reduction in PCR productivity when compared with a polio virus control.

^b Contamination levels exceeded 60,000 *E. coli* per 100 g shellfish flesh for both species.

^c Derived by logarithmic regression.

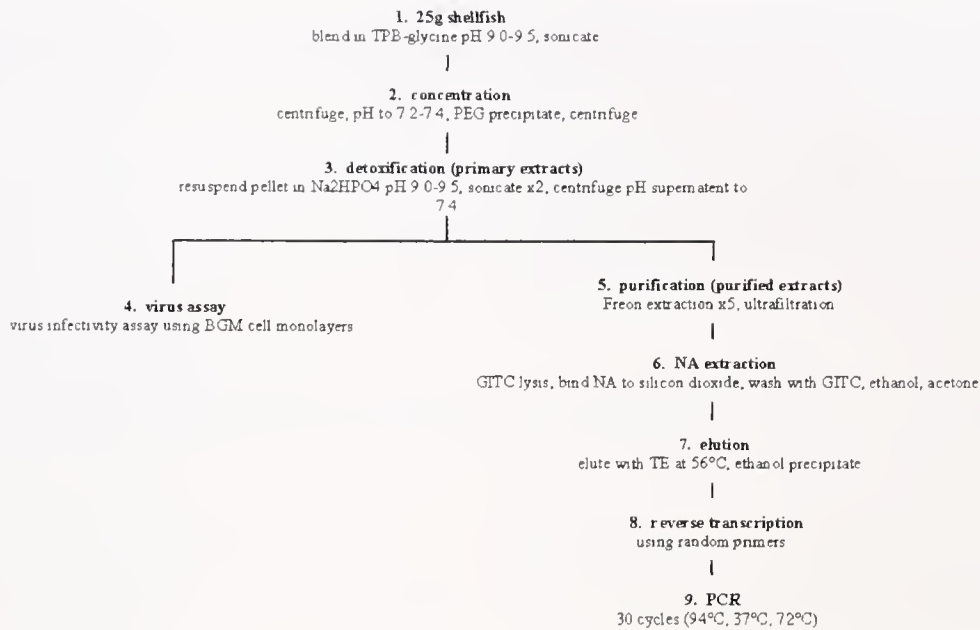


Figure 1. Outline scheme for extraction, purification, and amplification of enteroviruses from shellfish.

environmentally polluted shellfish were used as a substrate. Sample tolerances exceeded 6 g (equivalent weight) of shellfish in all cases. These results demonstrate that the shellfish processing procedures developed for removal of enterovirus PCR amplification inhibitors are also applicable to the SRSVs.

Field Samples

Environmentally contaminated oysters (*O. edulis* and *C. gigas*), mussels and cockles were obtained from several highly polluted field sites in the UK, processed through the virus and nucleic acid extraction procedures, and the SRSV PCR and Southern Blot hybridization was performed. Positive results were obtained in several samples indicating the presence of naturally occurring virus acquired through filter feeding in sewage-polluted waters. One sample was positive by both gel electrophoresis of PCR products and by Southern Blot at all shellfish concentrations tested. For other samples, specific PCR product was discernible only by Southern Blot hybridization and only when the highest concentration (7 g equivalent weight) of shellfish was tested. These results are consistent with those obtained in the seeding studies at low virus levels and probably indicate low virus titres in field samples.

Outbreak Samples

Following successful demonstration of SRSVs in shellfish from polluted field sites, shellfish associated with four incidents of human gastroenteritis were tested for the presence of SRSVs. PCR products were not demonstrable for any sample by gel electrophoresis. However, Southern Blot hybridization gave positive results for all samples tested. The majority of samples were positive by Southern Blot Hybridization at either the neat and 1:3 dilution but not at a 1:9 dilution. The absence of PCR product observable by gel electrophoresis and the titrating out of the majority of these samples by Southern Blot at a 1:9 dilution is similar to results obtained for polluted field samples. This, again, probably suggests that SRSV titers in such outbreak-associated shellfish are generally

low and that a single round PCR has inadequate sensitivity to routinely detect such levels.

Nested RT-PCR

Difficulty in routinely detecting SRSVs in shellfish by single-round PCR promoted the evaluation of a nested PCR (see Materials and Methods). This was evaluated using environmental and "outbreak" shellfish samples previously found to be only weakly positive by single-round RT-PCR. SRSV-specific amplicons were not visible by gel electrophoresis in any of the samples tested following single-round RT-PCR; however, all samples gave positive bands by nested RT-PCR. This shows that the nested RT-PCR was more sensitive, or less susceptible to inhibition, than the single-round RT-PCR. Moreover, nested RT-PCR amplicon bands were of a high intensity and, therefore, suitable for further characterization by sequencing.

Detection of SRSVs During Commercial Shellfish Production

Following development of the nested RT-PCR the procedure was applied to detection of SRSVs in commercially produced shellfish. Applications for commercial production included evaluation of the effectiveness of the RT-PCR for detection of virus contamination in "end-product" shellfish sold to the consumer and on the effectiveness of commercial shellfish purification for removal of SRSVs. These studies were performed on oysters (*Crassostrea gigas*) purified in a commercial processing plant associated with outbreaks of infectious disease during February and March 1996. "End product" monitoring of oysters from this particular plant during this period proved valuable for demonstrating batches contaminated with SRSVs. These findings were consistent with the available epidemiological data. Further monitoring of shellfish for SRSVs before and after purification during this period clearly showed that although the SRSV titer were reduced during purification (as judged by RT-PCR band intensity), the virus was not always completely cleared by this commercial processing.

DISCUSSION

Direct application of PCR to complex samples, such as shellfish, requires extensive sample purification, and a variety of protocols have been devised. The method we describe here employs a modified PEG virus extraction and concentration procedure (Lewis and Metcalf 1988) combined with further virus purification using Freon TF extraction, and finally, a nucleic acid extraction using a combination of glass-powder and guanidine isothiocyanate (Boom et al. 1990, Yamada et al. 1990). This novel approach to the extraction and purification of nucleic acid from enteric viruses in molluscan shellfish was found to largely overcome the RT-PCR inhibition problems associated with shellfish extracts.

Application of the shellfish extraction procedure to a PCR for the SRSVs yielded positive results in both environmentally polluted field samples and in shellfish associated with outbreaks of human infection. However, a feature of these results was the low incidence of positives containing enough amplified product to be visible by agarose electrophoresis alone. The majority of field results, and all outbreak-associated results, required visualization through the added sensitivity of Southern Blot hybridization. This was not a consequence of the methodology employed, because results were clearly visible by agarose electrophoresis during seed-

ing experiments. These results, and the tendency for samples to titrate out at a 1:9 dilution, suggest that SRSV titers in polluted shellfish are generally very low and that the single round PCR methodology was operating close to its limits of sensitivity.

Further seeding experiments demonstrated that a nested RT-PCR was significantly more sensitive for SRSV detection in shellfish extracts and also overcame any residual PCR inhibition associated with such extracts. The nested procedure was applied to monitoring aspects of commercial shellfish production. The nested RT-PCR proved capable of detecting SRSVs in processed shellfish sold for consumption from a commercial supplier associated with incidents of gastroenteritis resulting from oyster consumption. Further monitoring showed that although the commercial purification routinely applied to these oysters seemed to reduce virus content SRSVs were not reliably eliminated. The nested RT-PCR should prove valuable for further studies on the behavior of SRSVs during such commercial processes as purification and relaying. In addition, the procedure has applications for monitoring shellfish harvesting areas at risk of contamination with SRSVs, for investigation of SRSV contamination in the products of shellfish producers associated with outbreaks, and for direct investigation of shellfish causing illness.

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